Insulin Potentiating Activity of Cinnamomum Extract on 3T3-L1 Adipocytes

M. Taher[1] Fadzilah Adibah Abdul Majid[1] Mohamad Roji Sarmidi[1,2]

[1]*Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia*
Tel: +60-7-553884, email: muhammad_taher@yahoo.com

[2]*Chemical Engineering Pilot Plant, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia*

Abstract

Non-insulin dependent diabetes mellitus (NIDDM), or Type II diabetes is one of the largest health problems in the world. Several plant phytochemicals can directly stimulate insulin secretion and/or action. In this work, the activity of a chalcone polymer isolated from Cinnamomum zeylanicum on 3T3-L1 adipocytes was investigated. The compound was isolated using silica gel column chromatography. The result shows that at a dose of 100 µg/mL, the compound gave the higher action than insulin alone by increasing glucose consumption 3 hours after stimulation. When the compound was combined with insulin (100nM), the glucose consumption increased about 25 %. In contrast, when the compound was administered on preconfluent 3T3-L1 preadipocytes, there was no significant effect. Cell proliferation also increased after addition of the compound. Cell proliferation was detected by MTT assay. Based on the experimental data it can be shown that the compound promote glucose metabolism and subsequently enhanced cell growth. The type of action of the compound was synergic effect to insulin activity.

Key words:

Cinnamomum zeylanicum, Chalcone polymer, 3T3-L1, Cell proliferation, Glucose metabolism

Introduction

Non-insulin dependent diabetes mellitus (NIDDM), or Type II diabetes is one of the largest health problems in the World. Several plant phytochemicals can directly stimulate insulin secretion and/or action, and improve insulin action and binding.

Research into the health benefits of edible plants has been increasing during the last several years, driven by the consumers' awareness of the relationship between diet and optimal health. Recent scientific researches increasingly support the notion that components in foods and food ingredients have many potential health benefits. At present, the key to optimal health is considered to be high bioactivity-added groceries called nutraceuticals. The term nutraceuticals covers the gamut, including dietary supplements, functional foods, and medical foods. Consumption of a diet rich in plant foods will provide phytochemicals, non-nutritive substances in plants that possess health-protective benefits [1].

Cinnamon is the dried bark of several varieties of trees in the evergreen family of the *Lauraceae*. Scientific research is confirming cinnamon's traditional uses in disease prevention and its antimicrobial properties. *In vitro* studies conducted by the USDA's Agricultural Research Service (ARS) found that cinnamon's most active compound, methylhydroxy chalcone polymer (MHCP), increased glucose metabolism approximately 20-fold. If future research bears out this effect in humans, cinnamon may have the potential to delay or prevent adult-onset (type 2) diabetes. Further, MHCP prevented formation of free oxygen radicals in blood platelets. Studies have shown that antioxidants can slow the progression of some complications of diabetes. ARS is seeking a patent application on MHCP, the first chalcone (a type of polyphenol or flavonoid) reported in cinnamon. It was water soluble and not found in the spice oils used as food additives [2].

This paper reported the isolation of active compound from the bark of *Cinnamomum zeylanicum*. However, a complete spectroscopic data of the compound have not been yet obtained. Based on available data it could be concluded that the isolated compound was similar to methylhydroxy chalcone polymer that previously isolated by Taylor et al. [3]. At present, it is called chalcone polymer (upon confirming the structure). The chalcone polymer was tested for its activity on 3T3-L1 (preadipocytes and adipocytes) cell lines. Previous research by Taylor et al. [3] studied the phenomenon occurring inside cell by using radioactive chemicals. However this experiment monitored the phenomenon extracellularly by quantifying glucose concentration and cell proliferation.

Approach and methods

Materials

Chalcone polymer was obtained as described in the next section. Animal cell culture grade chemicals were obtained from Sigma Chemicals and Life Technologies (Gibco). 3T3-

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L1 was purchased from ECACC (No 86052701) and was grown as described in ECACC standard procedures.

Purification of Chalcone Polymer

The dried plants were purchased from the herbal market (Johor, Malaysia). The dried and powdered of the stem bark of *Cinnamonum zeylanicum* was extracted by soxhlet extractor for 18 hours with *n*-hexane, and acetone successively. The solvent for organic extract was removed under reducing pressure by rotary evaporator.

The acetone extract was separated by vacuum liquid chromatography on silica gel 230-400 mesh and eluted with EtOAc, acetone and MeOH to give fraction 1, 2 and 3 respectively. The combined fraction of 3 was evaporated by rotary evaporator and separated by column chromatography on silica gel 70-230 mesh and eluted with *n*-hexane: EtOAc (1:1), EtOAc, acetone and MeOH. The fractions were collected with test tube (25 mL) and the same R_f was combined to give fractions 1-5, 6-20, and 21-44. The combined fraction of 6-20 was separated by silica gel column chromatography and eluted with EtOAc and EtOAc: MeOH (8:2) afforded fraction of 4-10, 11-19 and 20-29. Fraction of 4-10 was washed with acetone to yield a pale brown amorphous solid (0.6403 g) and labeled as a chalcone polymer [4]. The compound reacted positively to FeCl3 with R_f of 0.8 in butanol: acetic acid: water (BAW, 4:1:5), m.p 205-207 °C (decomposed).

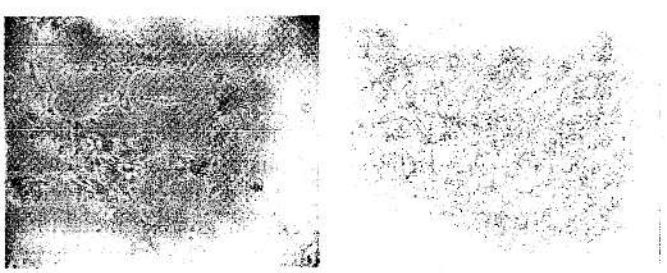

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Figure 1: Effect of 100 µg/mL of chalcone polymer on 3T3-L1 cell. (A) Picture of preconfluent 3T3-L1 preadipocytes two-days after treatment. (B) 3T3-L1 adipocytes after treatment on day 2.

Cell Culture

The 3T3-L1 cells were cultured and maintained as previously described by Taylor et. al [3] (2001) and Cheng [5]. Cell culture were propagated at 37°C in a humidified atmosphere of 5% CO_2 in DMEM containing 10% fetal bovine serum, 1 % penicillin (10.000 U/mL) and 1% streptomycin (10.000 µg/mL). Cells were seeded at 2-8 x10^4 into 24-well and 96-well plates for differentiation. Induction of differentiation was done one to two days post confluence. Cells were maintained in differentiation medium DMEM, 10% fetal bovine serum, 1% penicillin and streptomycin, 0.25 mM dexamethasone, 0.5 mM 1-isobutyl-3-methylxanthine (IBMX), and 1 µg/mL insulin) for four days, medium changed every two days. At day 4, the dexamethasone and IBMX were removed with insulin remaining on the cells for an additional two days. Differentiation was allowed to continue in DMEM supplemented with 10% fetal bovine serum. The sample was tested at day 9-14 post-induction. Prior to the cellular assays, cells were serum starved in DMEM for three hours, washed two times with KRPH buffer (5 mM Na_2HPO_4, 20 mM HEPES, pH 7.4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 136 mM NaCl, 4.7 mM KCl) and equilibrated 15 minutes in KRPH.

Glucose Analysis on Preconfluent 3T3-L1 Preadipocytes

Preconfluent 3T3-L1 preadipocytes were seeded in complete medium (10 % FBS and 1% penicillin/streptomycin) contained chalcone polymer at dose of 100 µg/m in 24-well plates at density of 2-8 x 10^4 cells per mL/well. Experiment was allowed to proceed for 3, 24, 48 hours. Supernatant was collected and analyzed by Biochemistry Analyzer (YSI 2700).

Glucose and Lactate Analysis on 3T3-L1 Adipocytes

The chalcone polymer at a dose of 100µg/mL was dissolved in complete medium. Insulin at dose of 100 nM was used as positive control. Control blank was without administering both of chalcone polymer and insulin. Experiment was allowed to proceed for 3 and 48 hours. Supernatant was collected and stored at –20 °C for further analysis. Glucose and lactate were analyzed by Biochemistry Analyzer (YSI 2700).

Cell Proliferation Analysis

The 3T3-L1 adipocytes were treated with concentration of 100µg/ml chalcone polymer. Experiment was allowed to proceed for 3, 24, and 48 hours at 37°C in humidified 5% CO_2 atmosphere. At the end of these periods, supernatants were discarded and cells were washed two times with phosphate buffer saline (PBS) then 20 µl of MTT stock solution (5 mg/ml) was added to each well and the plates were further incubated for 4 hour at 37°C and 100 µl of dimethyl sulfoxide (DMSO) was added to each well to solubilize the water-insoluble purple formazan crystals [6]. The absorbency was measured at wavelength of 570 nm and reference wavelength 630 nm with a microplate reader (Dynatech MR5000).

Results

Effect of Chalcone Polymer on Glucose Consumption

To determine whether chalcone polymer elevates glucose metabolism, 3T3-L1 adipocytes was cultured in the presence of 100 µg/mL chalcone polymer, insulin and mixture of both. Then glucose and lactate concentration were measured after 3 hours post inoculation.

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As shown in Fig. 2, the addition of chalcone polymer at the time of culturing increases glucose concentration. 3 hours after treatment, 100 µg/mL chalcone polymer elevated glucose metabolism by decreasing its concentration by nearly 80%. When the chalcone polymer was added together with insulin, glucose concentration decreases around 75% compared to control.

Lactate is a product of glucose metabolism in animal cell culture. After addition of both chalcone polymer and insulin on 3T3-L1 adipocytes, the elevating of lactate concentration was also increased. In Fig. 3, the lactate concentration was increased roughly 36 % compared to control at 48 hours post inoculation.

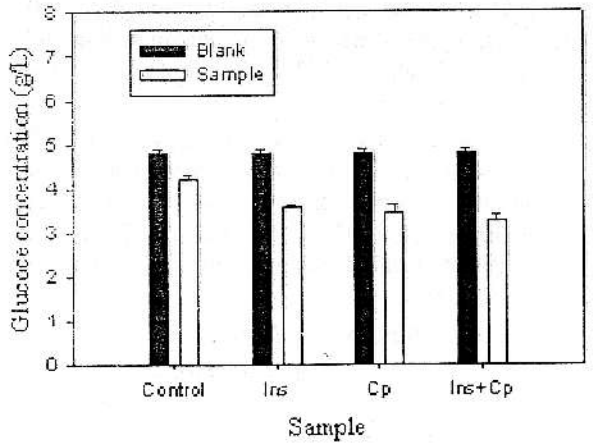

Figure 2: Effect of chalcone polymer on glucose concentration of 3T3-L1 adipocytes 3 hours after treatment. (Ins=insulin, Cp=chalcone polymer).

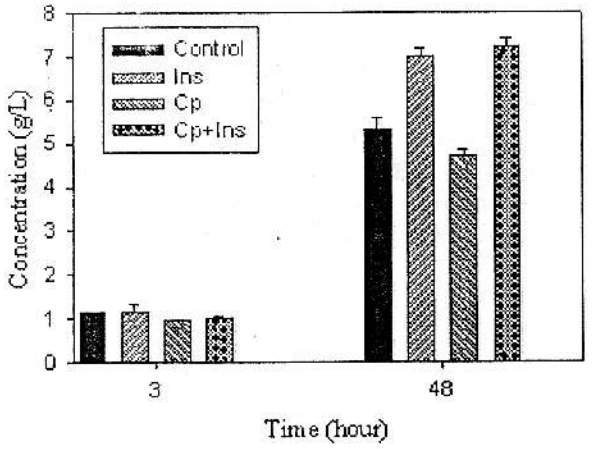

Figure 3: Effect of chalcone polymer on lactate production of 3T3-L1 adipocytes. Data shown reflect the means ± standard error of 2 experiments. (Ins=insulin, Cp=chalcone polymer).

Chalcone polymer promote cell proliferation

The presence of combination of 100 µg/mL of chalcone polymer and 100 nM insulin enhance the cell proliferation. The effect also depending on the time. Figure 3 shown that at 3 hours after treatment the activity was not significant. After 48 hours of treatment, the cell number of 3T3-L1 adipocytes was increase about 60 % of total number of the control tissue.

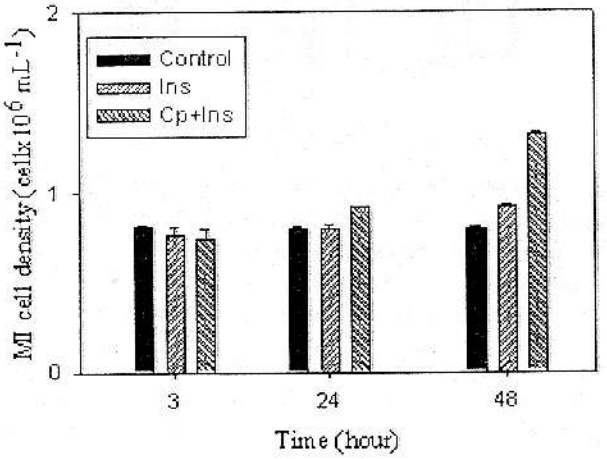

Figure 4: Effect of chalcone polymer on cell proliferation of 3T3-L1 adipocytes. (Ins=insulin, Cp=chalcone polymer).

Discussion

In this report, it was found that chalcone polymer alone could increase glucose metabolism on its own and more effective in the presence of insulin. In contrast, chalcone polymer did not give any effect on glucose metabolism of 3T3-L1 preadipocytes (Figure 5). The phenomena was probably due to preadipocytes express low levels of insulin receptor but high levels of insulin-like growth factor I (IGF-I) receptor, insulin appears to signal through the IGF-I receptor at the induction of differentiation [7].

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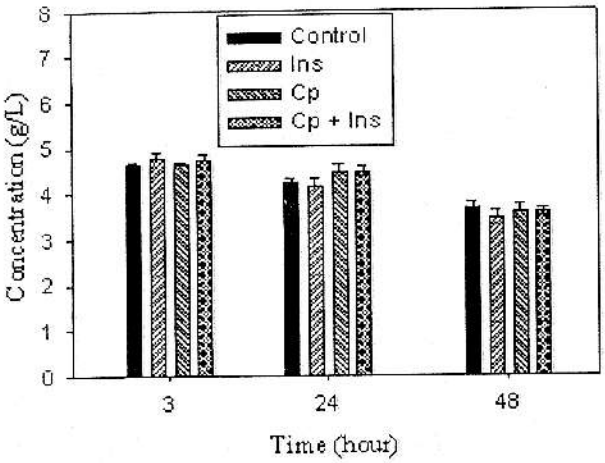

Figure 5 Effect of chalcone polymer on glucose consumption of 3T3-L1 preadipocytes. Preconfluent 3T3-L1 preadipocytes were treated with chalcone polymer 100 µg/mL, combination of chalcone polymer and insulin 100 nM and insulin alone as positive control. (Ins=insulin, Cp=chalcone polymer).

When investigating of the cell proliferation, it was found that chalcone polymer promote the growth of 3T3-L1 adipocytes. On other hand the compound inhibited cell proliferation when administered on 3T3-L1 preadipocytes, and producer cell lines (TB/C3 and CHO-K1) as described by Abdul Majid et al. [8]. Exposing of 3T3-L1 adipocytes to chalcone polymer increases cell number 24 hours after treatment. These finding demonstrate that chalcone polymer could promote the cell proliferation by potentiating insulin activity and subsequently promote cell growth. Interestingly, without addition of insulin, the chalcone polymer still active in promoting glucose metabolism. The activity of the compound was probably similar to insulin action and may be useful to apply for diabetic disease type 2.

Conclusion

Polyphenolic compounds including chalcone that exist widely in plants, could inhibit cell proliferation and increase cell differentiation in many cancerous and noncancerous cell lines. Terminal differentiation of preadipocytes to adipocytes depends on proliferation of both pre- and postconfluent preadipocytes, it was predicted that flavonoids would inhibit adipogenesis in the 3T3-L1 preadipocyte cell line [8, 9]. Chalcone polymer showed activity in 3T3-L1 adipocytes cell but inhibit cell proliferation when applied on preadipocytes cell line.

Acknowledgments

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