Activities of Jamu and Spices on Adipocytes Formation

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ABSTRACT

Jamu is a traditionally formulated herbs being used as traditional medicines for years to treat illnesses in the region of Indonesia and Malaysia. Different formulations are claims by the traditional healers/practitioners to cure or control many metabolic diseases such as hypertension, diabetes, cancer, stroke etc. In attempt to discover the activity of these traditional medicines that could promote adipogenesis, we investigated the ability of Jamu KM (traditional herbal preparation recommended for diabetes sufferers) extracts to stimulate 3T3-L1 preadipocytes. Extracts from Indonesian bay leaf (*Eugenia polyantha*) and cinnamon (*Cinnamomum zeylanicum*) which are previously claimed by scientific papers are also compared. In this study, we designed an experiment by replacing insulin with those extracts. The differentiated of 3T3-L1 adipocytes were monitored using oil red O staining method. Induction of adipocyte formation by those extracts gave the similar effects to insulin activity in adipogenesis.

Keywords: jamu, bay leaf, cinnamon extracts, adipocytes formation

INTRODUCTION

In Malaysia and Indonesia, cinnamon and bay leaves are widely used in kitchen as aromatic spices. These spices are also known in Indonesia as one of the ingredients in herbal traditional medicine for the treatment of diabetes. However, many of the traditional preparations have no scientific claims but patients' testimonials. Certain scientific explanations towards the remedial activities of the diseases could be explained using cell based assay studies. The approach in diabetes study in vitro uses many diabetes related cell lines. One of the most common cell lines used is adipocyte of 3T3-L1.

The biochemical pathways of adipogenesis have become increasingly well understood with the use of the 3T3-L1 model [1]. Treatment of cells with dexamethasone activates the transcription factor CCAAT/enhancer-binding protein β (C/EBP β). IBMX inhibits soluble cyclic nucleotide phosphodiesterases and results in increasing intracellular cAMP levels [2]. Treatment with IBMX results in activation of the related transcription factor C/EBP δ. Within 3 days of exposure to inducers, the cells undergo two rounds of mitosis [3]. Insulin promotes adipocyte differentiation by activating PI3-kinase and Akt activity [4]. Differentiation of adipocytes is accompanied by the accumulation of great

amounts of lipids in large cytoplasmic vesicles. A lipid-soluble red dye; Oil red O give a strong and bright staining of the lipid droplets in cytoplasm and is a reliable indicator of adipocyte differentiation.

The goal of this work is to evaluate the activity of cinnamon, Indonesian bay leaf and Jamu KM extracts in adipocyte differentiation. Preadipocyte culture was treated with different inducing agents and the lipid droplets accumulation was quantified. Oil red O assay was used to mark adipocytes differentiation in this experiment.

MATERIALS AND METHODS

Materials. 3T3-L1 cells obtained from ECACC**.** Dulbecco's modified Eagles medium (DMEM), fetal bovine serum (FBS) and other tissue culture reagents were purchased from GIBCO/BRL (Paisley, Scotland, UK). All chemicals used were purchased from Sigma (St. Louis, MO). Extraction of Cinnamtannin B1 and cinnamon water extract were carried out as described in Taher et al [5]. Jamu Kencing Manis was supplied by a traditional herbal medicine producer Millenium Multiherbs, Johor. Indonesian bay leaf was collected from the tree planted in UTM campus, Skudai, Johor. Dried cinnamon was purchased from the local spices shop in Pasar Larkin, Johor Bahru, Johor.

Extraction. Cinnamtannin B1 was isolated from *Cinnamomum zeylanicum* according to Taher [5]. Jamu KM and Indonesian bay leaf were extracted with food grade ethanol.

Adipocytes formation. Preadipocytes were cultured in 24 wells-plate for 4 days with two medium replacements as described in Taher *et al* [6]. The treatment was carried out by replacing medium with different preadipocytes inducers as indicated in Table I.

Samples		B (negative control	C (positive control)	1	2	3
Inducers added	Day 0	No inducer	Dex, 0.25 mM IBMX, 0.5 mM Insulin 1μg /mL	Dex, 0.25 mM IBMX, 0.5 mM cinnamtannin B1 100 μg/mL	Dex, 0.25 mM IBMX, 0.5 mM Bay leaf extract 150 μg/mL	Dex, 0.25 mM IBMX, 0.5 mM jamu extract 150 μg/mL
	Day 2	No inducer	Insulin 1μg /mL	cinnamtannin B1 100 μg/mL	Indonesian bay leaf extract 150 μg/mL	Jamu KM extract 150 μg/mL

Table I. Different type of inducers used for adipocytes formation. Dexamethasone (Dex), isobutylmethylxanthine (IBMX).

RESULTS AND DISCUSSION

In this experiment adipocyte differentiation was observed in all samples treated with different inducers as indicated in Table I. This result indicates that all the samples tested (cinnamtannin B1, Jamu KM and Indonesian bay leaf extracts) induced 3T3-L1 adipocyte cells differentiation. Figure 1A shows the formation of lipid droplets in all induced cells. Therefore it can be concluded that cinnamtannin B1, Jamu KM or bay leaf extracts can promote adipogenesis similar to insulin. This results support the previous finding [6]. Interestingly, extract of Jamu KM shows intensified lipid droplets in the tested cells. It was also observed that cells induced by Jamu KM formed lipid droplets after 5 days of induction in

comparison to the other samples. Jamu KM was formulated using combination of herbs, which is a proprietary formula. Currently, it is not known how this Jamu regulate cells differentiation and lipid formation faster than other inducers. Further bioassays on Jamu KM and Indonesian bay leaf extracts are currently under investigation for insulin receptor phosphorylation. Previous study has shown that CB1 promotes insulin receptor phosphorylation [5].

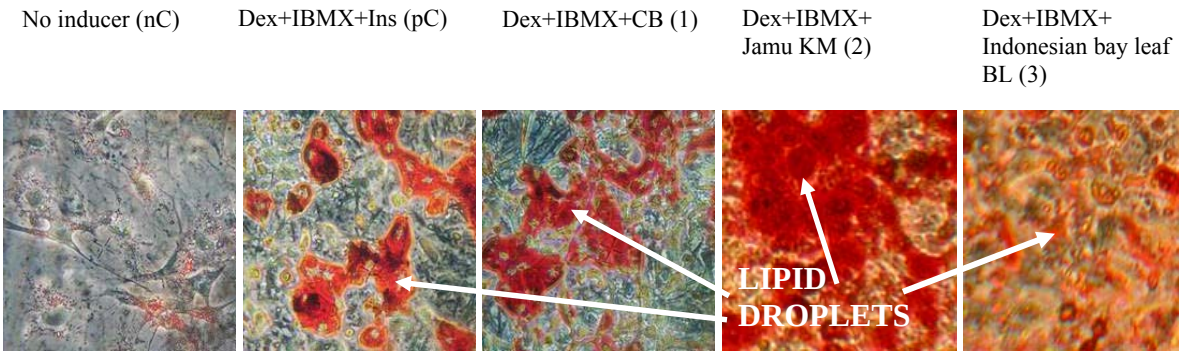

Figure 1. Effects of cinnamon extract on adipogenesis of 3T3-L1 preadipocytes. Differentiation is induced by addition inducers as indicated in Table I. After 11 days, preadipocytes differentiation was terminated and stained with oil red O. Note that lipid droplet can be seen in all the induced samples.

CONCLUSION AND SUGGESTIONS

This study shows that cocktails of herbs used in traditional herbal preparation for diabetes treatment able to act similarly to insulin in lipid droplet formation. Faster lipid formation in comparison to insulin induction could be due to other active compounds added in the formulation through unknown mechanisms. It is suggested that fingerprinting and profiling of the active compounds to be carried

out in this Jamu KM. Other aspect of metabolic mechanisms down regulated the diabetes activity in cells need to be investigated.

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