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ANTI-DIABETIC EFFECT OF *CURCUMA XANTHORRHIZA* ON ADIPOGENESIS OF 3T3-L1 PREADIPOCYTE

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ABSTRACT

Curcuma xantthorhiza (CX) also known as temu lawak in Malaysia is a herbal plant used widely in traditional medicine to treat various diseases including cancer, , cardiovascular disease and obesity. In the present study, water extract of CX demonstrated the ability to increase lipid accumulation and glucose consumption in mature adipocytes. The results suggest that water extract of CX has the potential to regulate blood glucose in diabetes mellitus patients.

1.0 Introduction

In the year 2020, statistical prediction by the Ministry of Health Malaysia indicates that a quarter of the Malaysian population will suffer diabetes mellitus. Even with the availability of modern drugs, many patients still relyon traditional medicine that consists of herbal preparation to treat diabetes. Countless research and development strategies were conducted by the government sector and private agencies to fulfill the demand. *Curcuma xanthoriza* (CX) or known as temu lawak in Malaysia is used widely in traditional medicine to treat various diseases including cancer, gastric, and diabetes. However its mechanism of action is still not well studied and documented. Adipose tissue is commonly used in biological research for diabetes mellitus and obesity studies to characterize lipid formation, glucose transport, insulin sensitivity and expression of adipocyte genes. This study investigates the anti-diabetic properties of WCX through the evaluation of adipogenesis in 3T3-L1 preadipocye and glucose consumption by adipocytes.

2.0 Materials and Methods

2.1 Plant extract preparation and cell differentiation

1.5 kg of raw CX was extracted in 20 liters of filtered water and spray dried. Pre-adipocytes differentiation was done according to Zebisch *et al*. (1) with modifications. Extract safe concentration screening: Preadipocytes were treated with a series of CX water extract (WCX) (10, 5, 1, 0.5, 0.1, 0.05, 0.01 and 0.005 mg/ml) for 24 hours. MTT solution was added and color developments were analyzed at 570 nm. Plates without treatments were used as a control. Safe concentration of WCX was selected for the following experiments.

2.2 Treatment and Oil Red O Staining (ORO)

Treatment of WCX was started at Day 0 of differentiation and finished at day 12. After treatment, cells were fixed and stained with ORO solution. Image was captured and color intensity was measured.

2.3 Glucose consumption

At day 12 of post differentiation, cells were serum starved overnight and then incubated in DMEM for 24 hours (2). Glucose was measured using COBAS C111.

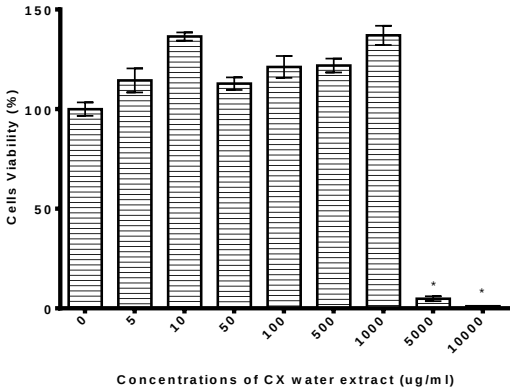

Fig. 1: MTT Assay. 3T3-L1 preadipocytes were exposed to WCX ranging from 5 µg/ml to 10000 µg/ml. Significant ($p < 0.05$) decrease in cell viability was observed at high concentrations (5000 and 10000 µg/ml). A concentration of 1000 µg/ml and lower was shown to benon-toxic to cells.

3.0 Results

3.1 Estimation of safe concentration

Preadipocytes were treated with a series of diluted WCX for 24 hours (Figure 1). Concentrations of 5000 and 10000 µg/ml significantly ($p > 0.05$) inhibited cell viability. On the other hand, concentrations of 5 to 1000 µg/ml were able to maintain high cell viability as per control ($p > 0.05$).

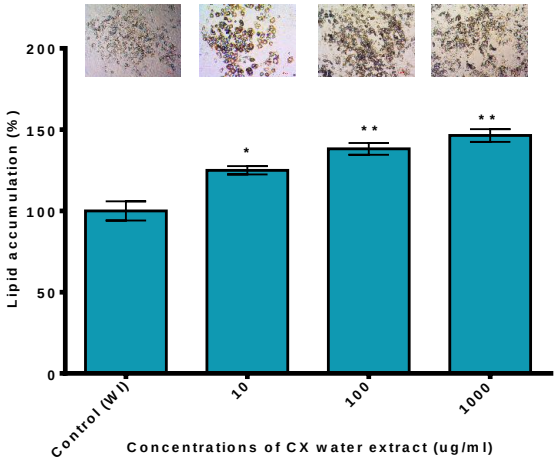

Fig. 2: Lipid accumulation. Significant difference were compared with control at *$p < 0.05$ and **$p < 0.01$ by Anova Test. Respective pictures above representing the formation of lipid in each samples.

WCX significantly promoted lipid accumulation up to ±150% compared to the control (insulin alone). Concentration of 1000 µg/ml displayed the highest accumulation with 146%. Meanwhile, 100 µg/ml and 10 µg/ml accumulated lipid up to 138% and 125% respectively. No significant difference ($p > 0.05$) of lipid accumulation between the three concentrations.

3.3 Glucose consumption

Three different concentrations of WCX showed significant reduction ($p < 0.05$) in glucose consumption in as compared to the control (insulin alone). Lower concentration of WCX consumed more glucose in the following sequence: 10 µg/ml (23.46 %) > 100 µg/ml (22.93 %) > 1000 µg/ml (19.73 %). No significant difference ($p > 0.05$) of glucose consumption between the three concentrations. In the absence of insulin, glucose consumption was significantly reduced ($p < 0.05$) by 3-fold.

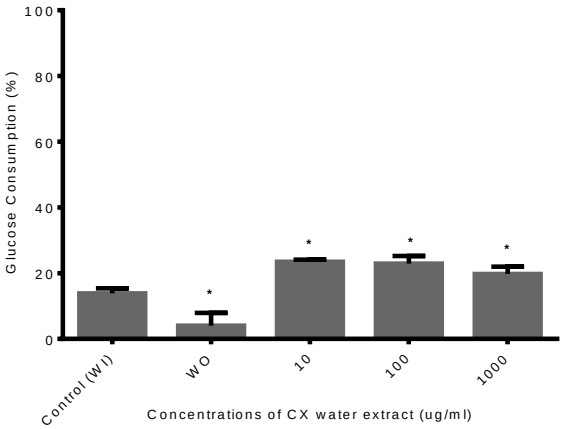

Fig. 3: Glucose consumption by mature adipocyte. 3-fold of glucose consumption was measured in control cells (insulin only) compared to non-induced cells.

4.0 Discussion and Conclusion

High blood glucose can be normalized by increasing glucose uptake into the cell. This activity is regulated by attachment of insulin on the insulin receptors (IRs) and induced by phosphorylation of glucose transports (GLUTs) from cytoplasm to cells membrane (3). Excess glucose in the blood is converted into lipid in adipocytes. In this study, WCX at concentrations between 10 to 1000 µg/ml demonstrated the ability to promote lipid formation and increase glucose consumption in the presence of insulin. An amount of 100 µg/ml WCX was selected as the most efficient concentration with significant effect on the amount of lipid accumulation and glucose consumption. *Curcuma xanthorrhiza* has shown to decrease blood sugar levels, total cholesterol, triglycerides and increase insulin secretion by activation of anion channel (4). In conclusion, WCX can be considered beneficial for preventing diabetes by increasing lipid accumulation and reduce the amount of glucose in media

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The authors wish to state that there are no conflicts of interest in this study.

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