UNIVERSITI TEKNOLOGI MARA

EVALUATION OF ANTIDIABETIC POTENTIAL OF METHANOLIC EXTRACT OF *Myrmecodia platytyrea* TUBER, *in vivo* AND *in vitro*

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ABSTRACT

Tubers of *Myrmecodia platytytrea* (Rubiaceae) has been used traditionally as an alternative therapy for the management of cancer and other inflammatory-related disorders. This plant is also believed to have the ability to lower blood glucose level. Nevertheless, no scientific proof is available on its anti-diabetic effect. Type 2 diabetes mellitus (T2DM) is one of the main non-communicable chronic diseases. Individuals present with T2DM have insulin resistance and usually develop insulin deficiency. The aim of this study was to investigate the potency of *Myrmecodia platytytrea* methanolic tuber extract (MPMTE) as an antihyperglycemic agent, *in vitro* and *in vivo*. Firstly, a simple and rapid high performance thin layer chromatography (HPTLC) technique was developed to measure the antioxidant (a direct HPTLC-DPPH• assay) and hypoglycemic effects (HPTLC with α-amylase) of *Myrmecodia platytyrea* tuber extracts (methanol, ethanol, dichloromethane and ethyl acetate extracts). Then, *in vitro* hypoglycemic effects via α-amylase and α-glucosidase inhibitory assays were conducted on the MPMTE. Cytotoxicity study of MPMTE was done against BRIN-BD11, 3T3-L1 and L6 using MTT assay before glucose uptake of MPMTE in these cell lines was measured by using Glucose Uptake Assay Kit while expression of glucose metabolism-related genes of cells were determined by quantitative RT-PCR. Finally, *in vivo* antihyperglycaemic effect of MPMTE was investigated for therapeutic and prophylactic treatments. Hyperglycaemia was induced in fasted SD rats with STZ (45 mg/kg; i.p.). In the therapeutic study, rats were treated orally with MPMTE (100, 200 and 400 mg/kg) and metformin (positive control, 100 mg/kg) daily for 14 days while in the prophylactic study rats were given MPMTE (100, 200 and 400 mg/kg) for 28 days before STZ induction. Blood was taken each week to measure fasting blood glucose level and at the end of experiment, for other biochemistry analysis. Our results showed that stigmasterol was detected in all extracts using HPLTC. The highest free radical scavenging activity was observed in the ethanol extract, which is rich in polyphenols and flavonoids. Additionally, MPMTE had both α-amylase and α-glucosidase inhibitory activities. MPMTE showed no cytotoxicity against BRIN-BD11, 3T3-L1 and L6 cells with IC_{50} values of > 500 µg/mL following 48 h incubation. Furthermore, MPMTE (125, 250 and 500 µg/mL) were able to downregulate expression of G6Pase gene and upregulate GCK, SREBP-1C, GLUT2 and GLUT4 genes against BRIN-BD11, 3T3-L1 and L6 cells, suggesting MPMTE regulated glucose metabolism. In the therapeutic study, STZ-induced diabetic rats treated with MPMTE (200 and 400 mg/kg) had significant decreased ($p < 0.05$) in fasting blood glucose, total cholesterol, triglycerides and low-density lipoprotein (LDL) with no significant changes in high-density lipoprotein (HDL) compared to STZ-induced untreated diabetic rats. Administration of MPMTE for 28 days prior to injection of STZ did not prevent the development of diabetes.In conclusion, MPMTE had strong antihyperglycaemic activity that inhibits glucose absorption in the intestine, modulate glucose metabolism, improving β-cell function, initiating insulin release and antioxidant, as well as anti-inflammatory effects due to the presence of high antioxidant compounds in MPMTE including polyphenolic acids, flavonoids and stigmasterol. Hence, MPMTE can be further developed into an adjuvant therapy for diabetic patients.

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CHAPTER ONE
INTRODUCTION

1.1 Background

Herbs and spices have been shown to have medicinal properties and many have demonstrated strong pharmacological activities (Bower *et al.,* 2016; Srinivasan, 2005). In controlled clinical trials on herbal medicines, 67% of those were reported to have statistically significant positive results (Pittler *et al.,* 2000). In 2010, it was reported that only 12% out of 1000 plants available in the market had scientific evidence of their efficacies i.e. the pharmacological and therapeutic application of only 356 plants has been published (Cravotto *et al.,* 2010).

Myrmecodia sp. is a plant that has a structure-like an anthill and ants (Lok and Tan, 2009). *Myrmecodia platytyrea* (*M. platytyrea*) (Rubiaceae) has been used as a medicine to treat tuberculosis, diarrhoea, haemorrhoids and ulcers. This plant is also used in cancer treatment, hyperuricaemia, and coronary heart disease. In addition, Myrmecodia is reliable in lowering blood glucose levels (Saptarini and Deswati, 2014). Therefore, this study aims to explain the likely mechanism of the hypoglycemic effect of *M. platytyrea* as an alternative therapy in the treatment of diabetes.

Diabetes is a disorder classified by hyperglycaemia, lack of insulin secretion and / or tissue sensitivity to insulin. Symptoms include polyuria, polydypsia, polyphagia and vision sometimes blurred usually accompanied by hyperglycaemia. Chronic hyperglycaemia results harmful effects such as kidney failure, neuropathy, blindness potential, and other cardiovascular disorders. There are three criteria for the diagnosis of diabetes, which are high fasting blood glucose, abnormal oral glucose tolerance test and the presence of symptoms of diabetes and hyperglycaemia. In short, in type 2 diabetes mellitus (T2DM), the body will react less or not at all to insulin. This is called insulin resistance. Insulin is produced by the body, but if the production is insufficient, glucose levels will increase. When the pancreas continues to produce more insulin, the function of insulin-producing beta cells (β-cells) will start to fail. People with T2DM may gradually lose their ability to produce insulin and as a result,