

# *In vivo* Antidiabetic and Antioxidant Potential of *Pseudovaria macrophylla* Extract

Aditya Arya, Hairin Taha, Ataul Karim Khan, Nayiar Shahid, Hapipah Mohd Ali, Mustafa Ali Mohd

**Abstract**—This study has investigated the antidiabetic and antioxidant potential of *Pseudovaria macrophylla* bark extract on streptozotocin–nicotinamide induced type 2 diabetic rats. LCMS-QTOF and NMR experiments were done to determine the chemical composition in the methanolic bark extract. For *in vivo* experiments, the STZ (60 mg/kg/b.w, 15 min after 120 mg/kg/1 nicotinamide, i.p.) induced diabetic rats were treated with methanolic extract of *Pseudovaria macrophylla* (200 and 400 mg/kg-bw) and glibenclamide (2.5 mg/kg) as positive control respectively. Biochemical parameters were assayed in the blood samples of all groups of rats. The pro-inflammatory cytokines, antioxidant status and plasma transforming growth factor  $\beta$ -1 (TGF- $\beta$ 1) were evaluated. The histological study of the pancreas was examined and its expression level of insulin was observed by immunohistochemistry. In addition, the expression of glucose transporters (GLUT 1, 2 and 4) were assessed in pancreas tissue by western blot analysis. The outcomes of the study displayed that the bark methanol extract of *Pseudovaria macrophylla* has potentially normalized the elevated blood glucose levels and improved serum insulin and C-peptide levels with significant increase in the antioxidant enzyme, reduced glutathione (GSH) and decrease in the level of lipid peroxidation (LPO). Additionally, the extract has markedly decreased the levels of serum pro-inflammatory cytokines and transforming growth factor  $\beta$ -1 (TGF- $\beta$ 1). Histopathology analysis demonstrated that *Pseudovaria macrophylla* has the potential to protect the pancreas of diabetic rats against peroxidation damage by downregulating oxidative stress and elevated hyperglycaemia. Furthermore, the expression of insulin protein, GLUT-1, GLUT-2 and GLUT-4 in pancreatic cells was enhanced. The findings of this study support the anti-diabetic claims of *Pseudovaria macrophylla* bark.

**Keywords**—Diabetes mellitus, *Pseudovaria macrophylla*, alkaloids, caffeic acid.

## I. INTRODUCTION

**D**IABETES MELLITUS (DM) is a complicated biological problem, in which the increased level of blood glucose is particularly due to lack of insulin or defective insulin activity. The inadequate insulin might heavily impact various biological activities, which includes metabolism of

carbohydrates, fats, proteins and electrolytes. Nevertheless, in long term usage, synthetic anti-diabetic drugs are more likely to produce side effects. Hence, an alternative approach from natural plant products is crucial for the treatment of diabetes. However, there were few documentation on the traditional and ethno botanical use of the medicinal plants and the effort of establishing the record is still on-going [10]. *Pseudovaria* species belong to the Annonaceae family of flowering plants of more than 130 genera, with approximately 2,300 to 2,500 species. Plants from this family are generally known as source for many types of alkaloids with many interesting pharmacological activities [15]. In Malaysia, *P. macrophylla* is locally known as *cagau biasa*, which can be found in the forest in Perak, Pahang, Terengganu including the Tioman Island [6]. It is traditionally used by the indigenous people known as *orang asli* to treat symptoms of cough and fever but little is known on its antidiabetic effect as it was not well documented [7]. Due to lack of sufficient literature on the role of this plant in diabetes, the current study was conducted to investigate the antidiabetic activity and the chemical constituents that may contribute to the activity.

## II. MATERIALS AND METHOD

Fig. 1 represents the materials and methods involved in this study. *P. macrophylla* was collected from the lowland forest area in Kenong Forest Park, Malaysia and then the dried stem bark of the plant was extracted with hexane and methanol followed by preliminary screening. The constituents present in the methanolic extract were analysed by LCMS, HPLC and NMR. After that *in vivo* study was carried out by determining the acute toxicity of the extract and inducing type-2 diabetes by STZ-Nicotinamide on SD rats and treating the rats with different doses for 45 days. Then blood glucose, serum insulin and C-peptide levels were measured along with oxidative stress markers, pro-inflammatory cytokines markers followed by histological analysis, immunohistochemical study and western blot.

Dr. Aditya Arya, Senior lecturer is with the Department of Pharmacy, Faculty of Medicine in the University of Malaya, Kuala Lumpur, 50603, Malaysia (corresponding author phone: +60379675749; fax: 303-555-5555; e-mail: aditya@um.edu.my, adityaarya18@gmail.com).

Hairin Taha and Professor Hapipah Mohd Ali are with the Department of Chemistry, Faculty of Science in the University of Malaya, Kuala Lumpur, 50603, Malaysia.

Md. Ataul Karim Khan and Nayiar Shahid are with the Department of Pharmacy, Faculty of Medicine in the University of Malaya, Kuala Lumpur, 50603, Malaysia (e-mail: ataul16@gmail.com, ataul16@gmail.com).

Professor Mustafa Ali Mohd is with the Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

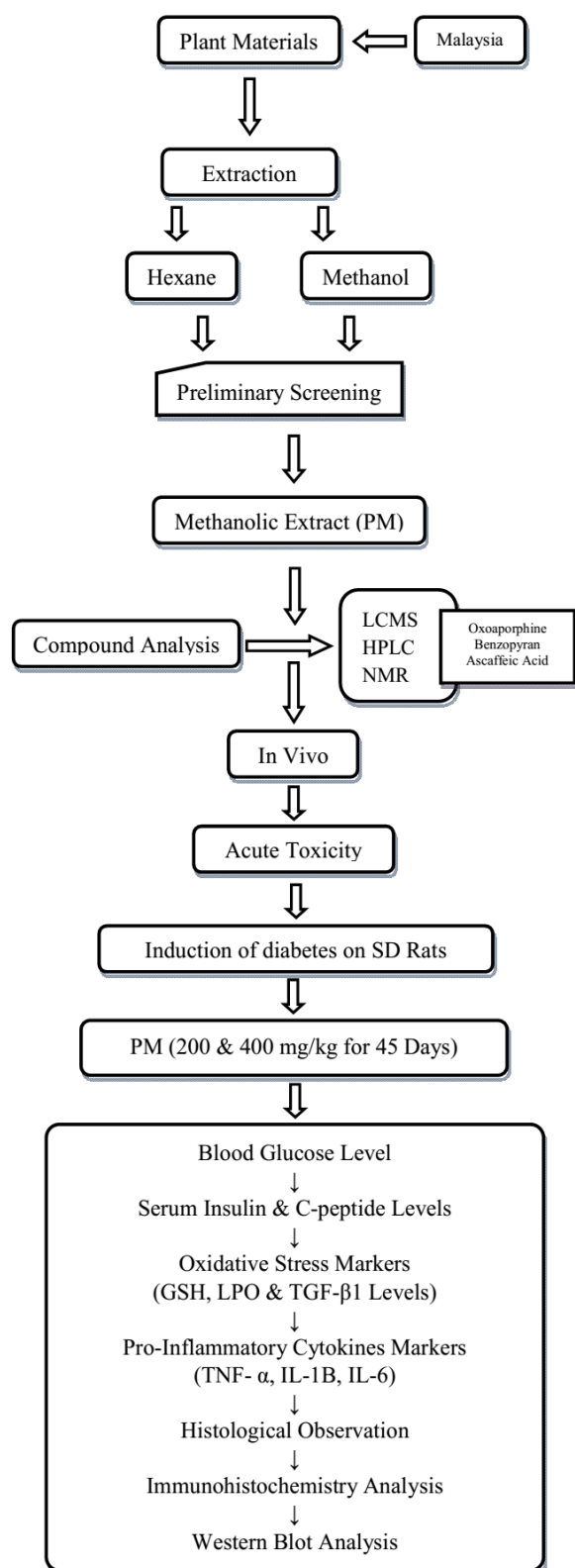


Fig. 1 Materials and methods of the study of *P. macrophylla* extract for determining antidiabetic and antioxidant potential

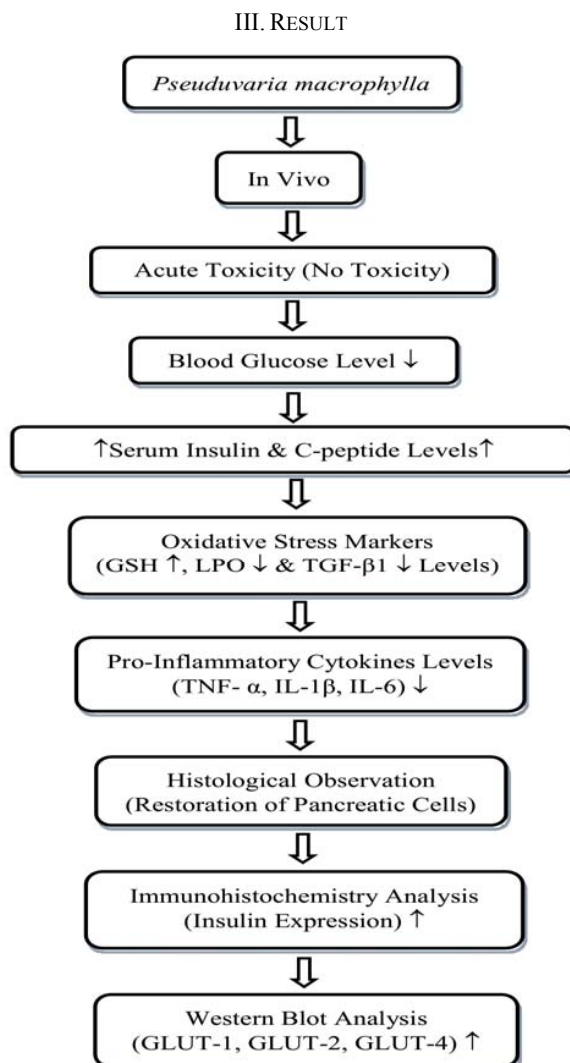


Fig. 2 Results of the study of *P. macrophylla* extract

Fig. 2 represents the results obtained in this study showing the antidiabetic and antioxidant properties of the methanolic extract of *P. macrophylla*. No acute toxicity was found with the extract and the blood glucose level was reduced with increase in insulin and C-peptide levels. The oxidative stress markers and proinflammatory cytokines levels were decreased with *P. macrophylla*. In the histology analysis, the degeneration of the pancreas was restored. The immunohistochemistry study showed increased insulin expression and western blot analysis demonstrated upregulation of GLUT-1, GLUT-2 and GLUT-4 proteins.

#### IV. DISCUSSION

Diabetes mellitus is a serious disorder that leads to several health problems and might ultimately shorten life span [5]. Researcher projected that three quarter of the global population might not be able to buy allopathic medicines so they use traditional medicines, particularly herbal products [3].

In this context, it has been proved that, few substances have potential anti-diabetic properties, which stimulate  $\beta$ -cell to secrete insulin and recover insulin sensitivity [8].

Phytochemical analysis on the bark methanolic extract of *P. macrophylla* revealed the presence of five major constituents; oxoaporphine alkaloids; lirioidenine **1**, O-methylmoschatoline **2**, phenolic acid; caffeic acid **3** benzopyran derivatives; polycerasoidol **4** and polycerasoidin **5** based on the peak intensity which could be responsible for the antidiabetic activity. One of the major compounds, polycerasoidol, which exhibited the strongest scavenging property on the DPPH radical similar to that of  $\alpha$ -tocopherol, could have contributed towards the antidiabetic activity. Our study revealed that *P. macrophylla* extract (200 and 400 mg/kg) has the capacity to lower blood glucose levels in STZ-nicotinamide induced diabetic rats and elevate the insulin level along with C-peptide levels significantly. Moreover, Jung has stated that, *P. macrophylla* is capable of substantially escalating the C-peptide levels in diabetic rats [4]. A previous study on medicinal plants proved that the presence of phenols and alkaloids contributed to the anti-diabetic activity of the plants [14].

The antioxidant property of methanolic extract of *P. macrophylla* bark has been demonstrated by increase in GSH and decrease in LPO level. It has been reported that, STZ induces severe oxidative stress in diabetic animals, which in turn, induces the peroxidation of polyunsaturated fatty acids, and lead to the formation of TBARS and MDA as by-products of LPO (lipid peroxidation) [9], [14]. In association with the positive impacts of *P. macrophylla*, we have also proved that it has potentially reduced the excessive oxidative stress marker (TGF- $\beta$ 1). Our results are in agreement with Rajavel who have found inhibition of (TGF- $\beta$ 1) in diabetic rats administered with palm oil, containing high level of phenolic compounds [11].

In this study, the phenolic compounds isolated from *P. macrophylla* bark have suppressed the secretion of pro-inflammatory cytokines in the diabetic rats treated with both doses. Based on these findings, it can be suggested that the ability of *P. macrophylla* to inhibit pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) may contribute to oxidative stress reduction. The pancreatic-cell disorder associated with insulin resistance is the symptom of type 2 diabetes [12]. However, both doses of *P. macrophylla* (PMa and PMb) effectively preserved islet and  $\beta$ -cell structure in our study. Thus, the extract restored the pancreas histology by alleviating oxidative stress which is in tune with [2].

Glucose is ingested into the cell through GLUT-2 and GLUT-4 in the plasma membrane of the cells. In pancreatic  $\beta$ -cells, glucose is the primary physiological stimulus for insulin secretion [13]. Our study suggested that, the modulation of GLUT-1, GLUT-2 and GLUT-4 protein could be one of the mechanisms of anti-diabetic activity. Thus, the anti-diabetic effect of *P. macrophylla* could be attributed to up-regulation of GLUT-1, GLUT-2 and GLUT-4 protein expressions, resulting in potentiation of pancreatic secretion of insulin from existing  $\beta$ -cells of islets. Similarly, the existed phenolic

compound in *Centratherum anthelminticum* seeds also enhanced insulin-signalling cascade, by increasing GLUT-2 and GLUT-4 expression, leading to increased glucose uptake in the pancreatic  $\beta$ -TC6 cells [1]. Therefore, the study reflects the anti-oxidant and anti-diabetic activities of *P. macrophylla* on STZ-nicotinamide induced rat models.

## V. CONCLUSION

Altogether, the study results conclude that *P. macrophylla* bark methanolic extract can be used in the management of diabetes by upregulating the antioxidant defense system and the expression of pancreatic proteins, whereas by downregulating the blood glucose and pro-inflammatory cytokines level.

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**Dr. Aditya Arya** is a Senior Lecturer at Department of Pharmacy, Faculty of Medicine, University of Malaya 50603 Kuala Lumpur, Malaysia. He is a member for many national and international scientific bodies, such as American Society for Pharmacology and Experimental Therapeutics, 2009 (ASPET). American Society of Pharmacognosy (ASP), International Committee. Registered Pharmacist.

Reviewer for many international (ISI) peer reviewed Journals: International Journal of Nanomedicine, Food and Function Journal, Food and Chemical Toxicology.

Areas of expertise: Drug Discovery (Natural Product Drug Discovery, Expert in Diabetes and Metabolic disorder research.