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In-vivo Blood Glucose Control by Phenolic Compound from *Vernonia amygdalina*, *Andrographis paniculata*, and *Pithecellobium jiringa* in Streptozotoxin-Induced Diabetic Rats

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ABSTRACT

Background: Phenolic compound from three plant species, *Vernonia amygdalina*, *Andrographis paniculata*, and *Pithecellobium jiringa* were extracted with 40, 50 and 60% ethanol in steam water bath shaker. The Gallic acid as phenolic compound representative was determined by HPLC. Among the three selected plants, *Pithecellobium jiringa* contains the highest amount of Gallic acid. The plant extracts were orally dosed to Streptozotoxin-induced diabetic rats. The rat's blood glucose was monitored for dose response toward Gallic acid. Significant amount of blood glucose reduction was found after 15 days of treatment of the plant extracts solution containing Gallic acid with the maximum of 31 % reduction shown by 25 mg/mL of *Pithecellobium jiringa dosed***.** Plant phenolic compound from *Pithecellobium jiringa* shows good potential for the treatment of diabetic Type 2.

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INTRODUCTION

1.1 Diabetes mellitus:

Diabetes mellitus is a chronic heterogeneous metabolic disorder caused by lack of insulin or reduced insulin activity that results in hyperglycaemia and abnormalities in carbohydrate, fat and protein metabolism. Estimated number of people with diabetes will increase from 382 million to 592 million by the year 2035 [1]. Diabetes will be the 7th leading cause of death in 2030 [2]. Diabetic complication may cause high glucose content in blood can reduce the blood flow in body, increase chance of foot ulcers, infection and eventually limb amputation if matters become worse [3]. Diabetes also can cause blindness, as an effect of damaged blood vessels in the retina [4]. People with diabetes have at least double risk of dying compared to their peers without diabetes [5]. Therefore, a study of effectiveness of natural remedies for diabetes will be useful for alternative treatment for the diabetic patient. The effectiveness is measured by monitoring blood glucose level of diabetic-induced rats, at a certain level of assumption. Diabetes-induced rats had been widely used for proving a wide range of natural products, in their ability to control the level of glucose in blood. Among the natural products are *Ajuga iva* [6] and *Suaeda fruticosa* [7]. Suitable dose of inducing Type 2 diabetes is 65mg kg-1 weight specimen Streptozotoxin by intraperitoneal injection [8]. The objective of the current work is to determine the Gallic acid content as the representative for phenolic compound in the selected plant species of *Vernonia amygdalina*, *Pithecellobium jiringa* and *Androghapis paniculata* in ethanolic solvent extraction and potential for inhibitor for metabolic inequilibrium of diabetic induced-rats. The potential for diabetic control was tested in dose response test to diabetic-induced white rats.

1.2 Phenolic compound:

Plant extracts contain phenolic compound which was known for its antioxidant property. There are many herbal species traditionally used to cure diabetic patients. In India, *Acacia arabica* is used for treating hyperglycemia [9]. *Vernonia amygdalina* has potential to be developed into medicine against diabetes and malaria [10] and was tested on obese rats to study the biochemical impact it has on the rats [11]. It is believed that phenolic compound contained in the traditional remedy is the antidote to reduce the glucose in blood level [12]. *Andrographis paniculata effectiveness* for the treatment of hypoglycemia was conducted by brewing the

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leaves with hot water [13]. Only one relevant article was found for *Pithecellobium jiringa* in aqueous form increases glucose level [14] which was contradict to the traditional believe that this species are able to reduce the blood glucose in diabetic patients.

MATERIALS AND METHOD

2.1 Material:

Selected plants used for phenolic compound extraction are *Vernonia amygdalina* (VA), collected from eastern part of peninsular Malaysia while *Androghaphis paniculata* (AP) and *Pithecellobium jiringa* (PJ) were collected from southern part of peninsular Malaysia. Ethanol solvent for extraction of phenolic compound in the samples was purchased from Merck with 99.8% purity. Streptozotoxin (STZ) from CalBioChem, U.S. with purity of 99.1% was used to induce the diabetic in rats. The 6 weeks white rats of Bulb-C were retrieved from local livestock agency. Gallic acid (GA) from R&M Marketing, UK at 99.5% was used in the phenolic compound determination.

2.2 Method:

All samples were dried and crushed using mortar and pestle until uniform size of >2.5 mm was achieved to increase surface area. The crushed sample was extracted at three different solvent strengths (40%, 50%, 60%) of ethanol and deionized water in a 500 mL flask at 20:1 solid to liquid ratio. The extraction was conducted in water bath shaker (INNOVA) for 4 hours at 60°C. The solvent was filtered using Whatman Filter No 41. The sample was refiltered with Nylon membrane 0.45 μm for HPLC analysis. An Agilent 1100 HPLC was used to determine the Gallic acid content in the extracted samples using isocratic elution in 50% methanol mobile phase using Zorbax SB-C18 column. Overnight-fasted rats were orally feed with a single dose of STZ at 65 mg/kg [6] which is dissolved in 0.9% sodium salt buffer. The three samples were orally dosed to the diabetic rats after 18 hours of STZ-induction. Control sample were monitored and kept at the same condition with the induced rats. Blood glucose level was measured using I-SEN glucose meter using microprocessor test strip from the tail-end puncture of the rats. Dose response test was conducted to four groups of rats, namely A, B and C for VA, PJ and AP, accordingly plus the control sample.

RESULT AND DISCUSSIONS

3.1 Gallic acid optimization:

The Gallic acid concentration of the extracted samples depicted in Figure 1 was determined based on the calibration curve of 5 to 500 ppm measured at 270 nm wavelength. PJ has the highest amount of Gallic acid extracted compared to the other 2 species. As a comparison, the amount of Gallic acid found in PJ is four times higher than in AP while, the amount of Gallic acid in AP is 2 times higher than VA. Among the 3 different solvent strength used to extract Gallic acid, 40% ethanol recorded the most optimum solvent strength for both of VA and AP. Meanwhile for PJ, the most optimum ethanol solvent strength is 50%. Lower solvent strength provides better elution of Gallic acid as phenolic compound is water soluble material.

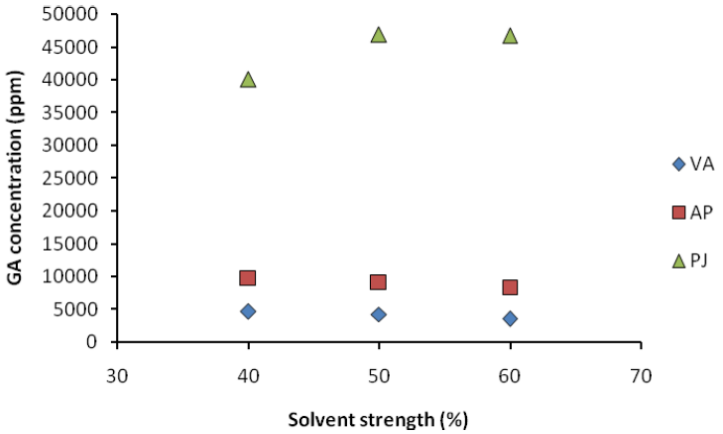

Fig. 1: Gallic acid found in *Vernonia amygdalina* (VA), *Androghapis paniculata* (AP) and *Pithecellobium jiringa* (PJ) ethanolic extracts.

3.2 Dose response test:

The initial blood glucose level was measured before STZ orally dosed to the rats at 65 mg/kg body weight at the end of the day one. Blood glucose was monitored on day two to observe the STZ effect before the rats are fed by the aqueous solution of the plants extracts except for control specimens. The control rats were fed with

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potable water without any treatment. All the rats are supplied with pellet food throughout the experiment. The STZ-induced rats were monitored for achieving blood glucose level above 200 mg/dL which the specimens were considered as diabetic Type 2. The percentage of blood reduction was counted when the rats are in diabetic condition measured against the final blood glucose level at day 15. Figures 2 and 3 show the blood glucose level and weight progress of the STZ-induced diabetes rats.

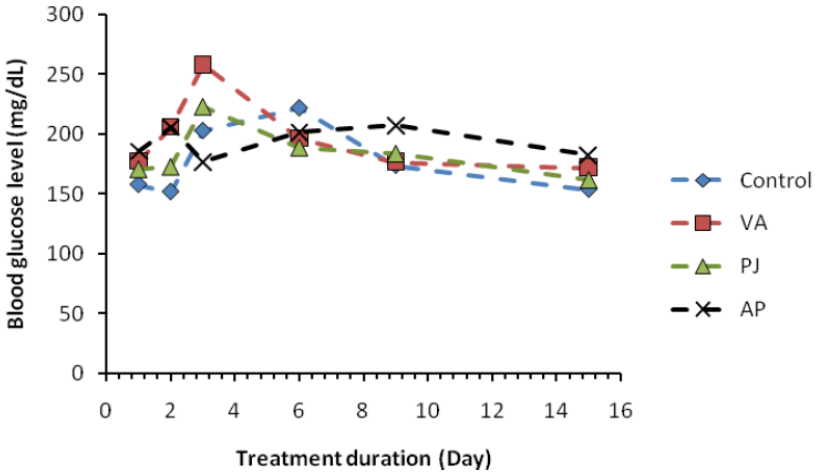

Fig. 2: Blood glucose level in STZ-induced rats from VA, PJ and AP-treated dose response.

The weight of the rats were monitored for monitoring glycemic effect on the mass body weight due to the treatment dosing. The weight gain of the rats were significantly observed in VA and AP-treated rats which was insignificant in the PJ-treated and control rats.

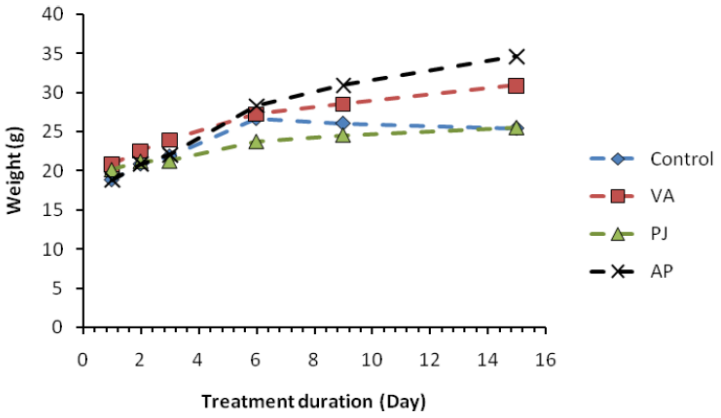

Fig. 3: Weight progress of the VA, PJ and AP-treated STZ-induced rats.

The remarkable blood glucose equilibrium was observed from PJ-treated specimen with 27 to 31 percent in blood glucose reduction, which was able to balance the blood glucose without any glycemic effect compared to the VA and AP-treated. The performance of PJ in the blood glucose equilibrium in the induced rats is contributed by the high content of Gallic acid found in the extracted sample. This finding shows the potential of PJ for the utilization of metabolic inequilibrium in diabetic case. The uptake of 1.0 mL aqueous extract based on 25 mg/mL crude extract which is equivalent to 1.25 mg/Kg dosed for the average initial body weight was able to show immediate effect of diabetic blood glucose control.

4. Conclusions:

The blood glucose equilibrium without giving any glycemic effect from PJ shows the use of alternative treatment from plant-based phenolic compound is a promising option to the current prescribed medicine used by the medicinal practitioner. The in-vivo test of plant extract in controlling blood glucose level on STZ-induced diabetes rats achieved maximum 31% reduction. The suitable dosed for human consumption in tablet form or liquid solution should be well estimated based on the real diabetic patients, Prediction of human dosage can be

calculated based on the in-vivo test conducted on the rats but human dose response may varied according to individual metabolic reaction, body weight and age.

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