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Antidiabetic Potentials of Leaves Extract of *Barringtonia Racemosa* (L) in Alloxan-Induced Albino Rats

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Abstract

Objective: Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by persistent hyperglycaemia and becoming a serious threat to mankind health in all parts of the world. This study was designed to study the methanol leaf extract potential of *Barringtonia racemosa* on Alloxan-induced diabetic.

Materials and Methods: The experimental rats were induced and made diabetic by single intra-peritoneal administration of Alloxan monohydrate at a dose rate of 160 mg/kg dissolved in 0.1 M freshly prepared citrate buffer at a base line pH 4.5. Extract of *Barringtonia racemosa* (100, 200, 300, 400 and 500 mg/kg). Group 1 (normal rats) were administered distilled water, Group 2 (Diabetic untreated rats) were administrated distilled water, while Group 3 received the standard drug (Insulin) at 0.1 mg/kg and the rest of the groups 5-8 were administered the methanol extracts. Blood glucose was determined by glucose oxidase method of Trinder.

Results: The blood glucose data obtained using Alloxan hyperglycaemic rats, dose of the methanol extract indicated a significant difference in the level of blood glucose with increase in the extract concentration at different days of administration ($p<0.05$) with 053.4 ± 5.5 and 045.5 ± 5.8 higher than the control 066.6 ± 5.2 at 400 mg/kg and 500 mg/kg post treatment.

Conclusion: The blood glucose data obtained using Alloxan hyperglycaemic rats clearly shows that the methanol extract of *Barringtonia racemosa* can produce significant and consistent hypoglycaemic effects.

Keywords: Antidiabetic; *Barringtonia racemosa*; Alloxan; Albino rats

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Introduction

Diabetes is a chronic metabolic disorder characterized by deficiencies in insulin secretion or insulin action associated with chronic hyperglycaemia and instability of carbohydrate, lipid and protein metabolism [1]. There are three types of diabetes mellitus recognized by the World Health Organization (WHO) such as:

(i)Type 1 diabetes (insulin-dependent)

(ii)Type 2 diabetes (non-insulin-dependent) and

(iii)Gestational diabetes

The β-cells in the pancreas are the key players in glycaemic homeostasis [2]. Diabetes mellitus causing significant mortality and morbidity. It is a serious debilitating and deadly disease that

has now reached epidemic proportions and the prevalence rates are expected to go even higher in the foreseeable future. It is a common disease in the developed and developing countries. According to a WHO report in 2011, approximately 360 million people globally suffer from diabetes. Diabetes epidemic is becoming pronounced in countries like Malaysia, Nigeria. As per reports of the WHO. It was reported by Mafauzy et al. [3] that the World Health Organisation (WHO) has estimated in 2030, Malaysia would have a total number of 2.48 million diabetics compared to 0.94 million in 2000 that is about 164% increase. It is expected that more people in Malaysia will be affected by diabetes in the near future.

Thus, management of this ailment diabetes in recent times and now possesses a big challenge. Apart from insulin, several types of glucose lowering drugs (including insulin secretagogues, insulin sensitizers, α-glucosidase inhibitors, peptide analogues, dipeptidyl peptidase-4 inhibitors and glucagon like peptide [4] have been developed. However, these synthetic oral hypoglycaemic agents have characteristic profiles of serious side effects, which include hypoglycaemia, weight gain, gastrointestinal discomfort, nausea, diarrhoea, liver, heart failure, etc [5]. Thus, to look into this menace, alternative therapy is the needed. Since centuries, many plants are considered to be a rich source of potent antidiabetic drugs and these herbal preparations are considered to be devoid of any side effects. It has been estimated that more than 400 plants and their secondary metabolites such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, tannins and polyphenolic derivatives are being used for the management of diabetes mellitus across the globe [6].

Therefore, the choice of *Barringtonia racemosa* a Malaysian mangroves unique plant communities growing at the interface between the land and sea in tropical as well as subtropical regions of the world was studied to curtly the danger of diabetic in Malaysia, Nigeria and the world at large.

The plant has highly developed morphological and physiological adaptations to the extreme conditions of their environment and possess metabolites of unique biological activity that are rich in medicinal potential. Traditionally, the plant is being used in folklore medicine for treatment of various ailments including diabetes [4]. However, the antidiabetic potentials of these plants are yet to be established. Hence, the present study on antidiabetic potentials of leaves extract of *Barringtonia racemosa* (L) in Alloxan-induced albino rats in management of diabetes with less complications and low cost effective.

Materials and Methods

Barringtonia racemosa were collected in Kampong Sarawak Malaysia by the river bank and Meranak at Meranak river bank in Kota-Samarahan Sarawak. Identification of the species was made by Prof Dr. Fasihuddin Bin Badruddin Ahmad and Prof Dr. Zaini B Assim. The samples were air-dried, cut into pieces and ground prior to analysis. It was then deposited into the polymer laboratory at Department of Chemistry, Faculty of Resource Science and Technology, UNIMAS.

Extraction method

The leaves of *Barringtonia racemosa* was extracted by the conventional solvent extraction method as described by Fasihuddin et al. [7]. This was achieved by soaking the ground plant material in non-polar, medium polar and polar solvents in the order of increasing polarity. A total of 2 kg of the dried and ground Leaves of *Barringtonia racemosa* was extracted using cold soaking method with hexane (C_6H_{14}). The samples were soaked in the hexane with the ratio of 1:3 in 5 litres Erlenmeyer flasks at room temperature for 72 hours. The resulting hexane solution was then filtered using filter paper and the residue was re-extracted with fresh hexane for another 72 hours and filtered. All the extracts were combined and concentrated using the rotary evaporator of model Heidolph Laborota 4000 efficient, under reduced pressure to obtain the hexane crude extract. The residues were then re-extracted using the same procedure with dichloromethane (CH_2CL_{l2}), then ethyl acetate (C_2H_5COOH), chloroform (CHC_{l3}), and methanol (MeOH) to obtain various extract of sample of dichloromethane, ethyl acetate, chloroform and methanol crude extracts, respectively. At the end of the extraction process the dry weight and yield of each crude extracts were determined.

In the procedure reported by Zeb et al. [8]. The methanol crude extract was dissolved in a small volume of methanol and added with petroleum ether to remove tannin. This was performed by adding the dissolved methanol extract into a separating funnel and then followed by adding the petroleum ether within the ratio 1:1. The sample in the separating funnel was shaken slowly and allowed to settle for 5 minutes until two layers of solution were clearly observed. At the end the petroleum ether extract was removed, allowing further re-separation of the methanol sample with petroleum ether. This process was repeated until the petroleum ether layer becomes colorless.

Chemical and reference drug

All chemicals and Drugs (Alloxan Monohydrate) used in this investigation were of analytical grade and were obtained from Sigma Chemical Co., St Louis, USA). Insulin (reference drug) was obtained from Medical Resource SDN BHD Kuching, Sarawak.

Experimental animals

Adult albino rats (Wister strains) weighing about 160-200 g body weight were used for this study. They were put in cages at room temperature (20-27°C) under 12/12 night/dark. They were maintained on a standard animal pellets (vital feeds, Grands cereals and oil meal) and water ad libitum for a period of one week. All the experiment was conducted based on the adherence to the ethical procedure on the use of animals for experiment.

Diabetes induction and extract administration

The experimental rats were induced and made diabetic by single intra-peritoneal administration of Alloxan monohydrate at a dose rate of 160 mg/kg dissolved in 0.1 M freshly prepared

Table 1 Effect of *Barringtonia racemosa* methanol extracts on mean glucose of diabetic rats.

Mean fasting blood glucose level post treatment (Days)							
Treatment groups	**Dose mg/kg**	**0**	**3**	**7**	**14**	**21**	**28**
Normal	0.25 ml	131.3 ± 11.3	123.5 ± 6.4*	122.6 ± 6.4*	119.4 ± 7.3*	120.0 ± 5.5*	121.2 ± 4.4d*
Negative	0.25 ml	414.3 ± 13.5	388.5 ± 5.3c*	411.7 ± 5.4c*	417.7 ± 5.9c	427.2 ±8.6d*	456.7 ± 6.4c
Positive	0.1 mg/kg	298.4 ± 4.5	143.5 ± 45.5	122.5 ± 3.6*	99.3 ± 3.4c*	076.3 ± 6.4	066.6 ± 5.2c*
Extracts	100	245.3 ± 12.3	226.4 ± 5.3*	198.7 ± 52.4c*	132.78 ± 15.3	124.6 ± 11.8	117.7 ± 9.7d
Extracts	200	285.6 ± 13.5	227.7 ± 7.7c*	211.5 ± 6.7c*	155.5 ± 4.4	133.3 ± 4.5	115.2 ± 8.5d
Extracts	300	255.3 ± 2.8	187.3 ± 5.9c*	135.9 ± 7.6c*	118.7 ± 7.4	105.8 ± 3.7	086.9 ± 6.4*
Extracts	400	157.6 ± 6.6	177.9 ± 1.3	101.8 ± 4.3	095.3 ± 7.3c*	082.4 ± 2.2	072.8 ± 3.6*
Extracts	500	398.6 ± 32.5	133.4 ± 3.5	090.8 ± 3.9	075.3 ± 2.7*	053.4 ± 5.5*	045.5 ± 5.8*

Value with superscripts c with a group along the row is significantly ($p<0.05$) higher than zero hours' blood glucose value with superscript d within the group along the row are significantly ($p<0.05$) lower than zero hours blood glucose value. While value with superscript (*) between groups along the column is significantly ($p<0.05$) lower than blood glucose value in the diabetic control group.

citrate buffer at a base line pH 4.5 as reported by Al-Shamaony et al. [9]. Baseline blood glucose was determined using glucose oxidase method, blood glucose level of more than 200 mg/kg was considered as diabetic. Animals were divided into eight groups of 5 rats each that had fasted for 24 h prior to receiving an oral dose of saline, (insulin, 0.1 mg/kg) and extract of *Barringtonia racemosa* (100, 200, 300,400 and 500 mg/kg). Group 1 (normal rats) were administered distilled water, Group 2 (diabetic untreated rats) were administrated distilled water, while Group 3 received the standard drug (Insulin) at 0.1 mg/kg and the rest of the groups 4-8 were administered the methanol extracts. Blood glucose was determined by glucose oxidase method of Trinder, using one Torch Basic Glucose monitoring system with little modification at 0, 3, 7, 14, 21, 28 days post extract of *Barringtonia racemosa* administration [10].

Statistical analysis

Data were expressed as Mean ± standard deviation for three determinations of each experiment. The analysis was done using the software-SPSS one-way ANOVA (**Table 1**).

Result and Discussion

The blood glucose data obtained using Alloxan hyperglycaemic rats, dose of the methanol extract. **Table 1** demonstrates the blood glucose experimental animals. There was a significant difference in the level of blood glucose with increase in the extract concentration at different days of administration ($p<0.05$).

The level of total hypoglycaemic increased during Alloxan-induced diabetes when compared with the corresponding groups. Administration of methanol extracts of *Barringtonia racemosa* at 100-500 mg/kg and values near normal at a dose of 500 mg/kg shows highly significant effect, when compared with the control (insulin). At days 21 and 28 at 500 mg/kg indicated higher significate rate with 053.4 ± 5.5 and 045.5 ± 5.8 higher than the control at 066.6 ± 5.2 post treatment. This agrees with the reported of Wang et al. [4] that *Barringtonia racemosa* inhibition of carbohydrate absorption and decreased gluconeogenesis and glycogenolysis.

This effect was as a result of the presence of several diterpenoids and triterpenoids which have been identified in *Barringtonia racemosa* extract as well as the pentacyclic triterpenoid, bartogenic acid, as the major active component of the plant [11,12]. It also agrees with our result that the methanol extracts as well as the pure compound of bartogenic acid from the plant was reported to inhibit the intestinal α-glucosidase activity. Thus, suppress the rise of blood glucose [13,14].

Thus, the significant and consistent hypoglycaemic effect of *Barringtonia racemosa* in diabetic rats after 28 days indicates that the plant extract acts by stimulating glucose utilization by peripheral tissues. These results confirm the earlier findings that glycosylated haemoglobin was found to increase in patients with diabetes mellitus as well as Regenerate β-cells of the pancreas in *Barringtonia spp* and the amount of this increase is directly proportional to the fasting blood glucose level [15-20]. We have observed a decrease in total haemoglobin during diabetes and this may be due to the increased formation of glycosylated haemoglobin. Our study also gave a clear view that *Barringtonia racemosa* prevents a significant elevation in glycosylated haemoglobin level in diabetic rats that were fed daily for 28 days with *Barringtonia racemosa.*

Conclusion

The blood glucose data obtained using Alloxan hyperglycaemic rats **Table 1** clearly shows that the methanol extract of *Barringtonia racemosa* can produce significant and consistent hypoglycaemic effects. Thus, the leaves caused significant reductions in diabetic drug-induced hyperglycaemia in albino rats, the current studies contribute to an insight of the prevailing perception of the efficiency of plant extracts commonly adopted in Malaysia as a dietary supplement or ancillary therapy for the preclusion and remedy of diabetes. Nonetheless, the evidence found so far has confirmed the efficacy of the plant extract of *Barringtonia racemosa* as an agent for antidiabetic. This extensive study can serve as an efficient mean for the nomination of plants extract having an intense potential for the unbolting of novel antidiabetic agents.

Elucidation and Characterization of the plant extracts are currently on-going in Natural product research laboratory University Malaysia Sarawak.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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