Research Article

Evaluation of \(\alpha\)-Glucosidase Inhibitory Effect of 50% Ethanolic Standardized Extract of Orthosiphon stamineus Benth in Normal and Streptozotocin-Induced Diabetic Rats

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In the present study, a 50% Ethanolic extract of Orthosiphon stamineus was tested for its \(\alpha\)-glucosidase inhibitory activity. In vivo assays of the extract (containing 1.02%, 3.76%, and 3.03% of \(3\)-hydroxy-5,6,7,4'-tetramethoxyflavone, sinensetin, and eupatorin, resp.) showed that it possessed an inhibitory activity against \(\alpha\)-glucosidase in normal rats loaded with starch and sucrose. The results showed that 1000 mg/kg of the 50% ethanolic extract of \(O.\) stamineus significantly \((P < 0.05)\) decreased the plasma glucose levels of the experimental animals in a manner resembling the effect of acarbose. In streptozotocin-induced diabetic rats, only the group treated with 1000 mg/kg of the extract showed significantly \((P < 0.05)\) lower plasma glucose levels after starch loading. Hence, \(\alpha\)-glucosidase inhibition might be one of the mechanisms by which \(O.\) stamineus extract exerts its antidiabetic effect. Furthermore, our findings indicated that the 50% ethanolic extract of \(O.\) stamineus can be considered as a potential agent for the management of diabetes mellitus.

1. Introduction

Type 2 diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, a condition which could either be attributed to insufficient insulin secretion or insulin resistance. The number of diabetic patients is rapidly rising in most parts of the world, especially in the developing countries such as Thailand, India, and Indonesia. Controlling blood glucose levels of diabetics within the normal range is mainly based on the use of oral hypoglycemic/antihyperglycemic agents and insulin. However, these conventional treatments have undesirable side effects [1–3]. Those shortcomings have led to a great interest in the use of medicinal plants as alternatives for the management of type 2 diabetes mellitus [4]. Control of postprandial plasma glucose levels is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications. Basically, a sudden rise in blood glucose levels, causing hyperglycemia in type 2 diabetic patients, would be due to starch hydrolysis by the \(\alpha\)-amylase and \(\alpha\)-glucosidases found in gastrointestinal tract [5]. Complex starches, oligosaccharides, and disaccharides must be broken down into monosaccharides (glucose and fructose) before they can be transported across the intestinal lumen (mainly in duodenum and upper jejunum) into the bloodstream and thereby increase blood glucose level. Thus, one of the effective strategies for the management of blood glucose level in type 2 DM is by inhibition of \(\alpha\)-glucosidases and \(\alpha\)-amylase [6, 7] which reduces the digestion of carbohydrates for production of monosaccharide and, hence, indirectly decreases blood glucose level. Among glucose lowering medications, \(\alpha\)-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks [8]. It was demonstrated that \(\alpha\)-glucosidase inhibitors could be used to prevent disorders such as diabetes, obesity, hyperlipidaemia, and hyperlipoproteinaemia [9]. Our previous study showed that 50% Ethanolic extract of \(O.\) stamineus and its active ingredient, sinensetin, were able to inhibit \(\alpha\)-glucosidase and \(\alpha\)-amylase in vitro [10]. To the best of our knowledge, there have been no other reports on in vivo \(\alpha\)-glucosidase inhibitory activity of \(O.\) stamineus. The present
2. Materials and Methods

2.1. Chemicals. Streptozotocin (Sigma Aldrich Chemical Co., USA), acarbose 50 mg (Bayer Pharmaceuticals, Leverkusen, Germany), starch (Ajax Chemicals, Sydney, Australia), sucrose and glucose (R & M Chemicals, Essex, UK), 3’hydroxy-5,6,7,4’-tetramethoxyflavone, sinensetin, and eupatorin (Indofine Chemical Company, New Jersey, USA) were purchased.

2.2. Plant Material and Extraction. Leaves of Orthosiphon stamineus were obtained from Kepala Batas, Pulau Pinang, Malaysia. The plant was identified at the School of Biological Sciences, Universiti Sains Malaysia, and a voucher specimen (10810) was deposited at its herbarium. The dried leaves were powdered using a milling machine and extracted with 50% ethanol at 55°C for 24 hours, 2 cycles) over a period of 6 days. The extract was filtered and concentrated at 40°C using a rotary evaporator (Buchi, Flawil, Switzerland). Finally, the concentrated extract was freeze-dried (Labconco Corporation, Kansas City, MO, USA) to yield a 10.3% of dry powder.

2.3. HPLC Analysis of the Plant Extract. HPLC analysis was performed using a Shimadzu-LC system (Shimadzu, Japan) equipped with a CBM-20A controller, LC-20AT pump, DGU-20A5 degasser, SIL-20A autosampler, SPD-20AV detector, and CTO-10ASvp column oven.

Chromatographic separations were achieved using an Agilent Eclipse Plus C18 (250 × 4.6 mm i.d., 5 μm). A Zorbax guard fittings kit packed with replaceable Eclipse Plus C18 Guard column (12.5 × 4.6 mm i.d., 5 μm) was used to protect the analytical column. A reverse-phase HPLC assay was carried out using an isocratic system with a flow rate of 1 mL/min and a column temperature equaling 25°C. As the mobile phase, a mixture of acetonitrile, isopropyl alcohol, and 0.02 M phosphate buffer (NaH₂PO₄) (30 : 15 : 55 v/v) was used with the pH adjusted to 3.5 using 85% phosphoric acid. The UV detection was set at 340 nm. The injection volume was 20 μL. Total run time was less than 20 min for each injection [11]. Data were acquired and processed with LC-Solution Software. The peaks were detected at 340 nm and identified using standard substances, namely, sinensetin, eupatorin, and 3’hydroxy-5,6,7,4’-tetramethoxyflavone. The 50% Ethanolic extract of O. stamineus was suspended. The sample was dissolved in HPLC mobile phase and was analyzed by HPLC. The 3. Results

3.1 Phytochemical Composition. HPLC analysis showed the percentages of 3’hydroxy-5,6,7,4’-tetramethoxyflavone, significant differences between the control and the experimental groups were determined using the LSD multiple comparison test. Differences with $P < 0.05$ were considered to be significant.
of the extract caused significant reduction in the blood glucose levels of glucose-loaded STZ-induced diabetic rats (Figure 4(b)). The acarbose-treated diabetic rats also did not show any significant reduction in the blood glucose levels.

4. Discussion

Diabetes mellitus is a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia with disturbances in carbohydrate, fat, and protein metabolism, resulting from defects in insulin secretion, insulin action, or both [13]. One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This is done by delaying the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong its overall digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial increase in plasma glucose [14]. Examples of such inhibitors in clinical use are acarbose, miglitol, and voglibose [15]. The reduction in the postprandial blood glucose levels caused by α-glucosidase inhibitors, such as miglitol and acarbose, following a starch load is well established. These agents act by inhibiting the last step in carbohydrate digestion, namely, the conversion of disaccharides to monosaccharides (glucose), resulting in a consequent decrease in the rate of entry of glucose into the systemic circulation.

Several classes of chemicals have been found in O. stamineus, proving it to be rich in flavonoids, terpenoids, caffeic acid derivatives, chromene, and phenolic compounds [16–18]. Some of the present phenolic compounds and flavonoids possess marked antidiabetic activities. Moreover, O. stamineus aqueous extract has been proven to exert antidiabetic and lipid lowering effects in diabetic rats [4]. Although there have been reports on the antihyperglycemic [4] and antidiabetic [19] activities of O. stamineus aqueous extracts, attributing their activities to free radical scavenging and, in part, to increased glucose metabolism, there have been no previous reports, at least to the best of our knowledge, on the in vivo activity of this plant extracts in relation to α-glucosidase and α-amylase inhibition.

This was the first study to evaluate the in vivo α-glucosidase inhibitory effect of O. stamineus extract in normal and STZ-induced diabetic rats. In the oral carbohydrate challenge tests, doses of 250 mg/kg and 500 mg/kg of a 50% ethanolic extract of O. stamineus did not reduce blood glucose levels after oral starch or sucrose loading either in normal or diabetic rats. The highest dose of 1000 mg/kg of the 50% ethanolic extract of O. stamineus reduced blood glucose levels after starch loading in both normal and diabetic rats. However, upon sucrose loading, the 1000 mg/kg dose only caused a blood glucose lowering response in normal rats but did not do so in diabetic animals. In the oral glucose tolerance test, 1000 mg/kg of the extract failed to show any significant blood glucose lowering activity either in normal or diabetic rats. Nevertheless, the above results for the 1000 mg/kg dose showed a striking similarity to the effects of acarbose. These findings also agreed with the previous studies in which 50%
ethanolic extract of *O. stamineus* and the isolated sinensetin compound showed inhibitory activity on \( \alpha \)-glucosidase and \( \alpha \)-amylase in vitro [10]. Acarbose caused a significant reduction in blood glucose levels upon starch and sucrose loading in normal and diabetic rats. In contrast, it failed to inhibit the increase in the blood glucose levels of glucose-loaded diabetic rats. \( \alpha \)-Glucosidase inhibitors are competitive inhibitors of the small intestinal \( \alpha \)-glucosidase enzymes that break down nonabsorbable oligosaccharides [20]. The most commonly used \( \alpha \)-glucosidase inhibitor of present so far is acarbose. Chemically, acarbose is an oligosaccharide produced by cultured strains of actinomycetes. It is a competitive inhibitor with a higher affinity for sucrose than glucoamylase and pancreatic \( \alpha \)-amylase [21]. The 50% ethanolic extract of *O. stamineus* seems to delay the rapid digestion of starch and sucrose, thus, lengthening the time needed for carbohydrate absorption. It may be that sinensetin or some other unknown compounds in the extract are responsible for this reduction in the blood glucose levels of the normal rats. The tendency of the 50% ethanolic extract of *O. stamineus* to suppress the increase in blood glucose levels of starch loaded diabetic rats suggests the involvement of \( \alpha \)-glucosidase inhibition. Acarbose-like drugs that inhibit the \( \alpha \)-glucosidases present in the epithelium of the small intestine, have been shown to decrease postprandial hyperglycemia [22] and to improve impaired glucose metabolism without promoting
insulin secretion in noninsulin dependent diabetes mellitus (NIDDM) patients [23]. These medications should be most useful for people who have just been diagnosed with type 2 diabetes and who have blood glucose levels that are only slightly above the level considered to be alarming for a diabetic. They are also useful for people who take a sulfonylurea (e.g., glibenclamide) or a biguanide (e.g., metformin) derivative as a sole medication and who would need additional medications to keep their blood glucose levels within a safe range. Therefore, delaying carbohydrate absorption with a plant-based α-glucosidase inhibitor such as *O. stamineus* extract offers a prospective therapeutic approach for the management of type 2 diabetes mellitus and may be beneficial for borderline diabetic patients [24].

Although this extract seems to be promising in the treatment of type 2 diabetes mellitus by reducing postprandial hyperglycemia, it is still too early to recommend its use in human. Only a thorough and full-fledged study could rationalize its use in human. The results suggest that the α-glucosidase inhibitory effect of the 50% ethanolic extract of *O. stamineus* may contribute to delaying carbohydrate digestion. Therefore, α-glucosidase inhibition could possibly be one of the mechanisms by which the 50% ethanolic extract of *O. stamineus* exerts its antidiabetic effects—indicating that *O. stamineus* could be considered as a potential drug in the management of NIDDM. The present results showed that *O. stamineus* extract significantly reduced the plasma glucose concentrations of sucrose-loaded normal and diabetic rats. Thus, *O. stamineus* extract may be beneficial for patients with diabetes mellitus.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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