Sapporo Medical Journal
Volume 56, Issue 06, June 2022

Antidiabetic Effect of Aqueous Extract of *Orthosiphon Stamineus* Benth on Alloxan-Induced Diabetic Rats

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Abstract— This study was conducted to determine the effect of oral extract of *O. stamineus* in the management of diabetes in normal and alloxan-induced diabetic rats. The rats were divided into four groups, with ten rats. Group A (control) consisted of normal rats that received 2 ml (10 ml/kg of body weight) of normal saline per day. In comparison, group B consisted of diabetic rats that received 1 ml (120 mg/kg of body weight) of treatment with extract of *O. stamineus*. On the other hand, Group C consisted of diabetic rats given 1 mL (150 mg/kg body weight) of metformin. On the other hand, Group D was made up of untreated diabetic rats who served as a negative control group. Alloxan (150 mg/kg body weight) was injected intraperitoneally into groups B, C, and D. Diabetic group B rats given *O. stamineus* extract had considerably lower blood glucose levels than diabetic group D rats ($p = 0.05$). Similarly, diabetic rats in group B consumed significantly less food and water than diabetic rats in group D ($p = 0.05$). After that, diabetic group B rats given *O. stamineus* extract had a considerable increase in body weight ($p = 0.001$). In conclusion, *O. stamineus* extract can reduce alloxan-induced hyperglycemia in diabetic rats and prevent weight loss in diabetic animals.

Keywords: Anti-diabetic, *Orthosiphon stamineus*, diabetic rats.

1. Introduction

Traditional medicine, as known, is the body of knowledge and practices used to recognize, prevent, and/or minimize some physical, mental, or social disorders that are based on experience and observation as it is passed down from generation to generation (in either verbal or written form). Complementary traditional medicines are used in impoverished nations [1, 2].

Diabetes is a category of metabolic illnesses characterized by high blood sugar (glucose) levels caused by abnormalities in insulin secretion or action, or perhaps both—insufficient insulin action results in elevated blood glucose levels. Diabetes mellitus is the most common metabolic condition (hyperglycemia) [3–5].

The inadequate action of insulin on specific tissues is the origin of abnormalities in glucose, lipid, and protein metabolism in diabetes [6]. Inadequate insulin action is caused by low insulin excretion or reduced tissue responses to insulin action at one or more stages along the intricate path of hormone function: insulin deficiency and delinquency or deficiency [7, 8].

Treatment of diabetes can be diet, exercise, oral hypoglycemic drugs, and insulin. Today, synthetic drugs are available and considered antidiabetic drugs, but they are expensive and can cause serious side effects. In addition to the treatment options available today, many herbs are used or adopted to treat diabetes. Medicinal plants have advantages because they do not have side effects [9, 10]. The current work estimates the antidiabetic activity of the water extract of *O. stamineus* in both the non-induction and alloxan-induced rats. Furthermore, this study demonstrated the direct effect of *O. stamineus* extract on insulin excretion in rats.

2. Results

As shown in Figure 1, weight was significantly increasedin group B (diabetic rats treated with *O. stamineus*) when compared to group D (control, untreated diabetic rats) at a level of significance ($p < 0.05$). Conversely, significant ($p < 0.05$) weight losses were recorded in group D compared to group A (normal rats) (Figure 1).

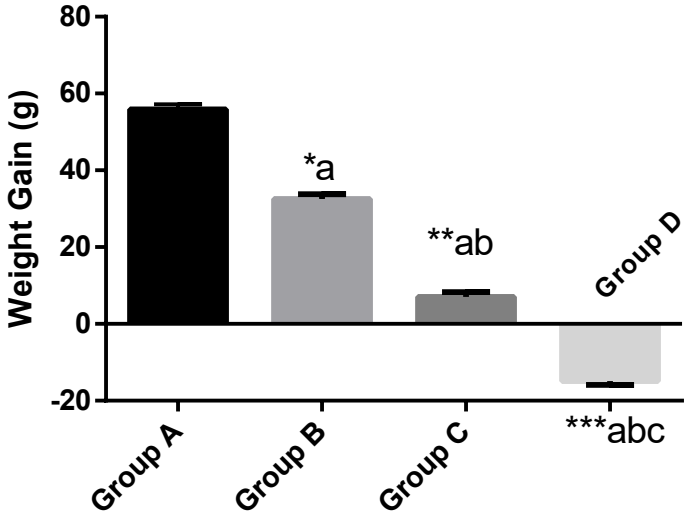

Figure 1. Weight gain for groups A, B, C, and D. A p-value < 0.05, 0.01, and 0.001 for groups B, C, and D, respectively, indicating more weight loss. Statistically significant difference was considered present at $P < 0.05$, $n = 10$.

As presented inFigures 2 and 3, the water and food intake significantly ($p < 0.05$) increased in the diabetes-induced alloxan groups (B, C, and D) compared to group A (regular group). Significant decreases in all feeding behavior parameters, water, and food consumption were found after treatment with the aqueous extract of *O. stamineus* compared to untreated diabetic rats. The water and food consumption were significantly ($p < 0.05$) enhanced in group D (untreated diabetic rats) compared to group A (normal rats) as a control group. Quantitative value reductions ($p < 0.05$) in water and food intakes were found after *O. stamineus* extract and metformin treatment.

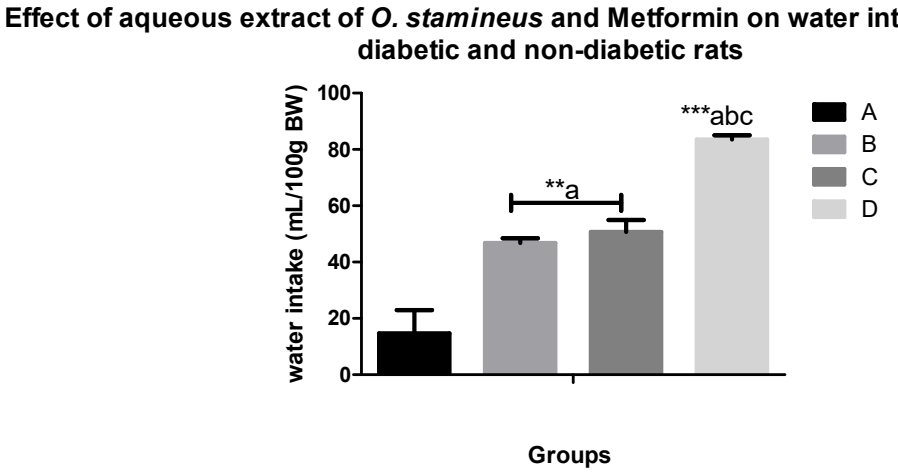

Figure 2. Comparisons of water intake in various groups. There was a significant increase in groups B and C (p-value < 0.01), it was tremendous in group D (p-value < 0.001)

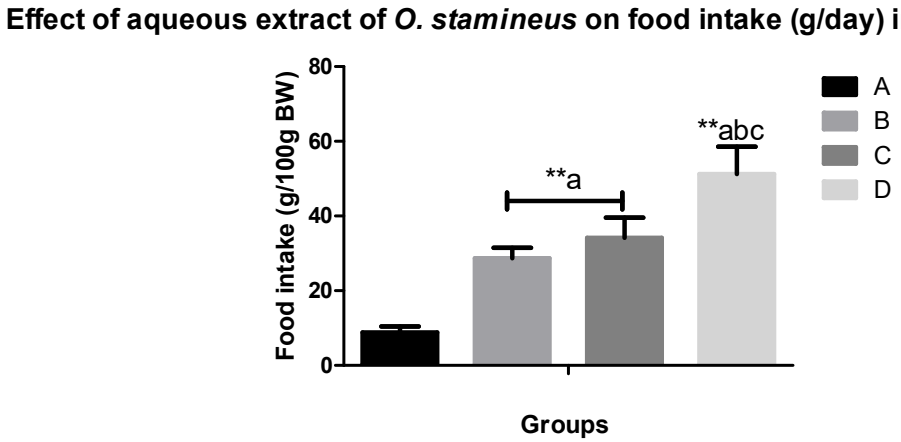

Figure 3. Comparisons of food intake in various groups. Food intake was increased in groups B, C (p-value < 0.01), and D (p-value < 0.001).

The blood glucose significantly increased in Alloxan-induced diabetic rats (groups B, C, and D) compared to group A (normal rats) ($p<0.05$). A statistically significantly lower ($p<0.05$) in

blood glucose levels was observed in the groups treated with *O.stamineus* extract (group B) and metformin (group C) compared to group D (control diabetic rats).

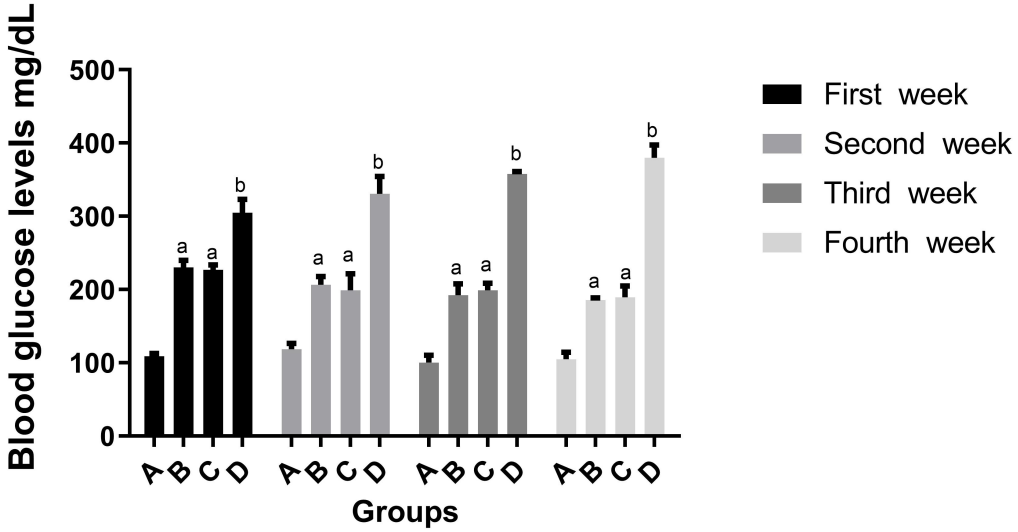

Figure 4. Comparisons of blood glucose levels in various groups. (a) indicates a significant value ($P \leq 0.05$) in contrast to group A. (b) shows a significant value ($P \leq 0.05$) incomparison to other groups.

Insulin levels in group D (untreated diabetic rats) showed a significant decrease in comparison to the values in group A (normal rats) ($p< 0.05$) (Figure 5-A). At the same time, the levels of glucagon were significantly increased ($p < 0.05$) in diabetic rats (Figure 5-B). In addition, treatment with *O. stamineus* (group B) and metformin (group C) enhanced serum insulin significantly ($p < 0.05$) when compared to group D (untreated diabetic rats). On the other hand, glucagon levels were significantly lower ($p < 0.05$) after oral administration with *O. stamineus* and metformin than those in untreated diabetic rats.

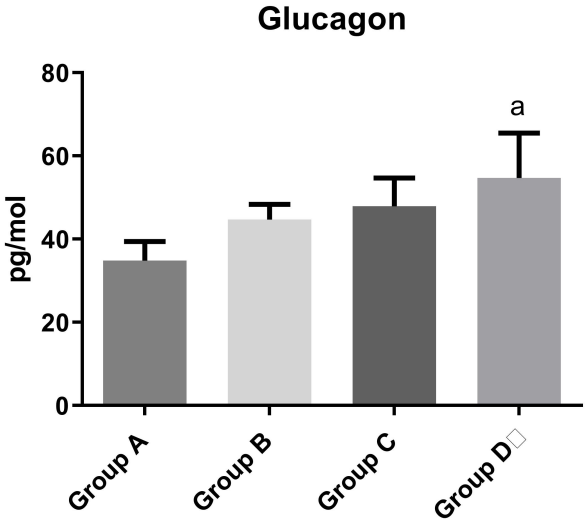

Figure 5-A. Comparisons of insulin levels in various groups. (a) indicates a significant value ($P \leq 0.05$) compared to groups A and B.

Table 5: Mean values of serum insulin in diabetic rats

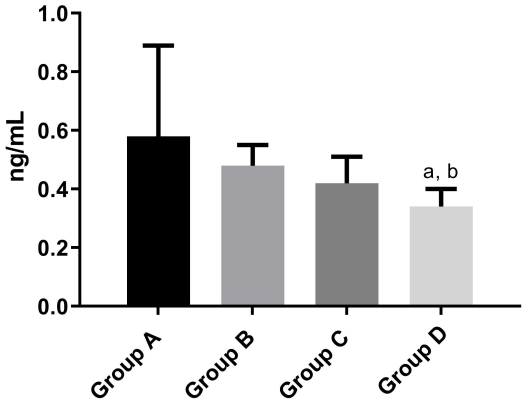

Figure 5-B. Comparisons of glucagon levels in various groups. (a) indicates a highly significant value ($P \leq 0.01$) in contrast to group A. (b) shows a significant value ($P \leq 0.05$) in comparison to group B.

Altogether, insulin levels in group D (untreated diabetic rats) significantly decreased, but glucagon levels increased in diabetic rats. Treatment with *O. stamineus* (group B) and metformin (group C) enhanced serum insulin greatly ($p < 0.05$) when compared to the level of normal rats.

3. Discussion

Several plant species with hypoglycemic activity have been identified and reviewed extensively in the literature; most of these plants contain bioactive compounds such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, and so on, which are frequently implicated in having an anti-diabetic effect [11–15]. Among them is *O. stamineus*, which is used in traditional medicine used in the treatment of diabetes and chronic renal failure through the Inhibiting α-amylase and α-glucosidase activities, antioxidant and anti-inflammatory activities, regulating lipid metabolism, promoting insulin secretion, facilitating insulin resistance, increasing glucose uptake, promoting glycolysis, inhibiting gluconeogenesis, promoting glucagon-like peptide-1 (GLP-1) secretion, and antiglycation activity, which was the main mechanisms of *O. stamineus* in the treatment of diabetes and its complications. The main components for hypoglycemia effects in *O. stamineus* may be phenolic acids, flavonoids, and triterpenoids [16, 17].

Alloxan induces diabetes by destroying the insulin-secreting cells of the pancreas, resulting in hyperglycemia[18]. Observations through the current research correlated with the former study's findings that the blood glucose level increased significantly in alloxan-induced diabetic rats. Thus, one of the most potent procedures to induce chemical diabetes mellitus by alloxan, which causes an increase in blood glucose and decreases insulin production [19].

Diabetes is described by polyphagia and polydipsia, clear from the increased food and water intake reported in the diabetic group of animals [20]. However, the results of this research revealed a significant ($p < 0.05$) reduction in body weight after the administration of alloxan in comparison to group A (normal rats) (Figure 1). In the experimental duration, the body weight was decreased in diabetic rats induced by alloxan. In contrast, there was a significant increase in body

weight in group B (treated with *O. stamineus* extract) compared to group D (untreated diabetic rats). The capacity of the aqueous extract to restore body weight appears to affect its ability to decrease hyperglycemia[21]. In this study, body weight reduction may be due to increased urine production, causing dehydration [21].

The water and food consumption in diabetic rats treated with the extract of *O. stamineus* were significantly decreased compared to untreated diabetic rats (group D). They were comparable with group metformin (C) for the whole duration of experimentation ($p < 0.05$). Actions of the free radicals could cause repeated damage to the ß-cells of the pancreas, causing a progressive decrease in insulin secretion, which may lead to the exaggeration of hyperglycemia and its complication ($p < 0.05$).

The current research findings showed a significantly raised blood glucose level after induced diabetes compared to normal rats ($p < 0.05$). The treatment with the aqueous extract of *O. stamineus* (group B) and metformin (group C) was capable of decreasing blood glucose levels significantly compared with diabetes groups ($p < 0.05$). Our findings are consistent with other study findings. It was observed that blood glucose was reduced because of either a more significant increase of insulin or the release of bound insulin[21].

Hypoglycaemic effects of *O. stamineus* can result from increasing marginal glucose use. Inhibiting the proximal tubular re-absorption mechanisms to maintain glucose in the kidney could also participate in glucose-lowering effects[22]. The hypoglycaemic activities might perform general mediation by enhancing the peripheral absorption of glucose and increasing insulin secretion, decreasing the glucagon secretion, or might be because of the intestinal decrease of the absorption of sugar [23].

Our results can be explained by the ability of *O. stamineus* treatment to enhance *O. stamineus* treatments, to enhance partial regenerations and proliferation of pancreatic β-cells in alloxan-inducing rats, which causes a higher rise in insulin secretions and causes a reduction in glucagon secretions [21, 23].

4. Materials and Methods

4.1 Collection and Preparation of Plant Material

O. stamineus was obtained from a local nursery (Pak Ali nursery) in Skudai Johor, Malaysia. They were identified by Prof. Dr. Jumaat Haji Adam, Faculty of Science and Technology,Universiti Kebangsaan, Malaysia. The plants have been cut into small pieces and dried at room temperature for four days. The Plants (100 g) were boiled in 400 mL of distilled water for 15 min. The extracts were filtered using filter papers, and the resulting filtrates were kept in a deep freezer at -80ºC overnight. The filtrates were dried using a freeze drier (Labconco, U.S.A.) for four days, and the freeze-dried plant extracts (yield 10%) were kept in a desiccator for subsequent analysis. Plant extract solutions were prepared by adding 1 g of freeze-dried powder to 1 L of distilled water[24–27].

4.2. Animals

Forty male Wistar albino rats weighed (150–200 g) were obtained from the animal house, Science college, Yarmouk University, Jordan. Animals were divided into five groups in each cage and kept under a controlled environment of Temperature (22±2) O C, humidity (55±5) %, and a 12-hour dark/light cycle. All rats were kept in typical metal open cages and were free access to standard chow and water ad libtum.

4.3. Induction of diabetes

To induce diabetes, alloxan monohydrate (B.O.H Chemical Ltd, U.K.) was administered intra-peritoneally (200 µl) to fasting rats for 18 hours before collecting blood samples. [22, 28].

4.4. Experimental Design

Forty rats were divided into four groups for the experimental group, with five rats in each cage: Group A consisted of 10 rats (regular group). Rats were administered two mL daily of normal saline (10 mL/kg bwt) orally for four weeks [29, 30]. Group B: This group consisted of 10 rats (diabetic rats). This group was administered daily with 1 mL of *O. stamineus* extract (120 mg/kg bwt) for four weeks[21, 25]. Similarly,Group C consisted of 10 rats (diabetic rats). Rats received 1 mL of reference medication, metformin (150 mg/kg bwt), for four weeks. While Group D: This group consisted of 10 rats (diabetic rats as control).

4.5. Collection of Blood

Blood analysis was performed as described previously [31]. Blood samples (approximately 50 µL) were drawn from the tail vein using heparinized capillary tubes for plasma insulin determination. Blood samples (about 250 µL) were taken from the orbital sinus with heparinized capillary tubes to determine active plasma GLP-1. To perform the assay, blood samples were centrifuged at 7,000 rpm for 5 minutes at 4°C. Enzyme-linked immunosorbent assay kits (Ultra-sensitive rat insulin ELISA kit; Morinaga Institute of Biological Science, Kanagawa) and insulin (Morinaga Institute of Biological Science) were used to measure plasma insulin concentrations. A Glucagon-Like Peptide (Active) ELISA kit measured plasma immunoreactive intact GLP-1 levels (Linco Research, St. Charles, MO, USA). The other part of the blood was placed plane tube and then centrifuged at (400 rpm for 10 min, Acceleratedfor nine, and deaccelerate five) for biochemical tests [22, 28].

4.6. Statistical analysis

The results are demonstratedas mean ± standard deviation (S.D.). Data was carried out by one-way ANOVA followed by Tukey's multiple comparisons test for statistical analysis throughout the study. GraphPad Prism 6.0 software (La Jolla, CA, USA) was used for analysis. A statistically significant difference was present at $P < 0.05$ [32, 33].

5. CONCLUSION

In conclusion, the current study shows that *O. stamineus* extract can significantly reduce alloxan-induced hyperglycemia and prevent weight loss in diabetic animals. This effect is partly explained by the increase in serum insulin and the decrease in glucagon plasma levels. Therefore, current

research results also support the potential use of *O. stamineus* extract as a treatment for hyperglycemia.

Acknowledgment

The authors acknowledge the financial support provided by the UTM research grant GUP Tier 1 (Vote: 03H13), FRGS (vote: 2F126), Ministry of Higher Education (MOHE), and R.M.C. Ethical committee approval of this study number 27045128 according to Yarmouk University.

Conflict of interest statement

The authors declared no conflict of interest.

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