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RESEARCH ARTICLE

Does *Orthosiphon stamineus* Benth. enhance GLUT4 translocation in the skeletal muscle of induced type II diabetic rats?

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ABSTRACT

Background: GLUT4 acts as the insulin-responsive glucose transporter, thus promoting the uptake of glucose from circulation into muscle and adipose tissue. **Aims and Objectives:** The present study was conducted to determine the effect *Orthosiphon stamineus* Benth. extract on GLUT4 translocation in the skeletal muscle of type II diabetic rats using immunofluorescent technique. **Materials and Methods:** Male Wistar rats aged 10 weeks (180–220 g) were divided into four groups: Normal control group (vehicle receiving group), positive control group (diabetic rats treated with 5 mg/kg metformin), negative control group (diabetic rats not receiving any treatment), and treated group (diabetic rats treated with 1 g/kg *O. stamineus* Benth. aqueous leaf extract). The rats were treated daily for 14 days where the fasting blood glucose level was measured daily; meanwhile, serum insulin was measured before (after injected with STZ-NAD) and after they get treated (at the end of 14 days). At the end of the treatment, the rats were sacrificed and soleus muscles were dissected, fixed in 10% buffered formalin, and processed for immunofluorescence technique. **Results:** The findings showed significantly reduced ($P < 0.01$) mean fasting glucose concentration in positive control and treated groups. Relative pancreas weight and serum plasma concentration were similar in all groups. In the diabetic rats treated with *O. stamineus* Benth., improvement in the translocation activity of GLUT4 was observed. **Conclusion:** Our findings suggest that *O. stamineus* Benth. has the potential in the treatment of Type II diabetes mellitus though the improvement of GLUT4 translocation activity.

KEYWORDS: Diabetes Mellitus; GLUT 4; Immunofluorescent Technique; Metformin; *Orthosiphon stamineus* Benth

INTRODUCTION

Insulin-stimulated glucose transport into skeletal muscle is important in glucose homeostasis. Deviations in insulin-mediated glucose utilization are responsible for Type II diabetes mellitus which can be due to the development of insulin resistance or the impairment of insulin secretion from the beta cells in the Islet of Langerhans.[1] The development of insulin resistance has been linked to the impairment of glucose transport across the peripheral tissues, in particular, the skeletal muscle.[2]

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GLUT is a glucose transporter protein which is expressed in insulin-sensitive cells such as adipose tissue, skeletal muscle cells, and cardiomyocytes. GLUT isoform resides mainly in the intracellular membrane compartment under low insulin condition and is translocated to the plasma membrane on stimulation by insulin.[3] To date, 14 glucose transporter's isoforms have been identified and one or more of these GLUT isoforms are expressed in almost every type of human cell, with more expression of GLUT4 identified in the human skeletal muscle.[4-6]

The translocation of GLUT4 involves various steps and alterations at any of the steps could disrupt GLUT4 translocation to the plasma membrane, resulting results in impaired glucose transport. In case of skeletal muscle, GLUT4 expression was not affected in Type II diabetes mellitus, but the translocation pathway is mainly affected.[7] Inhibition of one of the proteins in the translocation signaling pathway such as phosphatidylinositol-3 kinase is shown to prevent the translocation of GLUT4 from the intracellular membrane to the plasma membrane.[6] This molecular defect, compounded by the impairment of insulin secretion by the pancreatic beta cells, leads to Type II diabetes mellitus.[8]

Orthosiphon stamineus Benth. is among popular plants used by Malaysian as an alternative treatment for diabetes mellitus due to its ability to lower glucose levels in the blood. Previous phytochemical analysis of *O. stamineus* showed the presence of sinensetin, eupatorin, and 3′-hydroxy-5, 6, 7, 4′-tetramethoxyflavone which are believed to be responsible for the antidiabetic effect of the plant. Flavonoids, terpenoids, saponins, hexoses, organic acids, caffeic acid derivatives, chromene, and myoinositol are the other phytocompounds present in *O. stamineus*.[9] Animal study has shown that the aqueous extract of this plant has hypoglycemic and antihyperglycemic effects on both normal and diabetic rats.[10] Moreover, while a reduction in blood glucose level was observed, a fraction from *O. stamineus* Benth. chloroform extract was shown to have no stimulatory effect on insulin secretion, suggesting that the observed reduction in blood glucose concentration was due to an extrapancreatic mechanism.[11] Therefore, the aim of this present study is to determine the effect of *O. stamineus* Benth. aqueous leaf extract on GLUT 4 translocation in the skeletal muscles of diabetic rats.

MATERIALS AND METHODS

Animal Preparation

Male Wistar rats (10 weeks old, 180–220 g) were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. All the experiments were conducted according to the protocols approved by the Animal Ethics Committee of Universiti Kuala Lumpur - Royal College of Medicine Perak. All rats were housed in plastic cages (where food and water were provided *ad libitum)* and maintained in a controlled environment (12 h light/dark cycle; 24–26°C). These rats were allowed to acclimatize for 7 days to laboratory space before the experiment.

Plant Material and Extraction Preparation

O. stamineus Benth. dried leaves were obtained from a state - run herbal garden Taman Herba, Perak. The aqueous extract was prepared based on the method by Rahim and Khan, 2006. Briefly, a 100 g of the course powder were boiled in 1 L distilled water and filtered. The filtered extract was dried overnight in an oven at 50°C and stored at −20°C until further use.

Induction of Diabetes

Induction of diabetes in rats was conducted according to the method of Rao *et al*.(2013)[12] Animals were considered diabetic if their fasting blood glucose concentration was more than 11.11 mmol/L.[13]

Experimental Design

The rats were divided into four groups (n = 7–8): Normal control group (vehicle receiving group), positive control group (diabetic rats treated with 5 mg/kg metformin), negative control group (diabetic rats not receiving any treatment), and treated group (diabetic rats treated with 1 g/kg *O. stamineus* Benth. aqueous leaf extract). The experiment was conducted for 14 days. Fasting blood glucose level was measured at day 0 and day 14 using a glucometer. At the end of day 14, the rats were sacrificed by cervical dislocation under anesthesia. Soleus muscles were dissected, weighed, and fixed in 10% buffered formalin as preparation for immunofluorescence staining of GLUT4.

Data were analyzed using IBM SPSS (version 23). Paired sample t-test was used to determine the difference of the fasting blood glucose level over the experimental period. $P < 0.01$ was considered to be statistically significant.

RESULTS

Fasting Blood Glucose Level

The fasting glucose level was measured at day 0 and 14 of the treatment. At day 0, all the three diabetic groups: Positive control, negative control, and treated groups were shown to have high mean fasting glucose level which was above 11.11 mmol/L (positive control group (16.61 ± 1.36), negative control group [16.33 ± 1.46], and treated group [21.78 ± 1.44]) when compared to the normal group (7.27 ± 0.91). As shown in Figure 1, the mean fasting glucose level was significantly reduced ($P < 0.01$) in the positive control and treated groups (positive control [7.18 ± 0.34]; treated group [9.35 ± 0.72]) after 14 days of treatment. However, the negative control group did not show any reduction in glucose level (16.50 ± 1.76) when compared with the normal group (6.14 ± 0.23).

Immunofluorescence Staining GLUT4

GLUT4 translocation in the adult rat soleus muscles of the normal and experimental groups was studied using the immunofluorescence technique. In the normal, positive control, and treated groups, the GLUT4 was seen at the soleus muscle fibers plasma membrane as fine scattered structures which appear as a diffuse background stain as shown in Figure 2a,c,d. However, the GLUT4 was less observed at the plasma membrane of the soleus muscle fibers in the negative control group as shown in Figure 2b.

Relative Pancreas Weight

No significant differences in relative pancreas weight were seen among the groups as shown in Table 1. According to Table 2, no significant difference in serum insulin concentration was observed between the treated group and the other groups.

DISCUSSION

This study was conducted to determine the effect of *O. stamineus* Benth. aqueous leaf extract on GLUT4 translocation in the skeletal muscles of diabetic rats using immunofluorescence staining. Skeletal muscle plays a major role in maintaining the homeostasis of blood glucose. Development of insulin resistance has been linked to the impairment of glucose transport across the peripheral tissues, in particular, on skeletal muscles.[14] In response to insulin, GLUT4 plays the role as a glucose regulator in skeletal muscle tissue where GLUT4, which is produced intracellularly, is translocated within the cell to the plasma membrane to facilitate glucose transport into the cell.

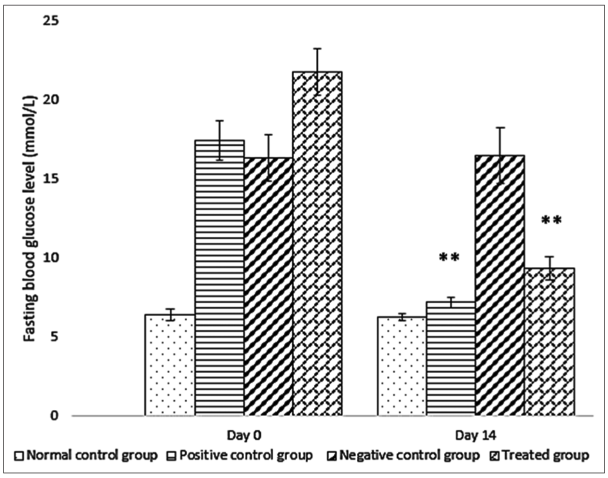

Figure 1: The effects of oral administration of *O. stamineus* Benth. leaves aqueous extract (1 g/kg) after 14 days fasting blood glucose level of metformin-induced diabetic rats. The values are presented as mean ± standard error of the mean with (**) indicates statistically significant difference ($P < 0.01$)

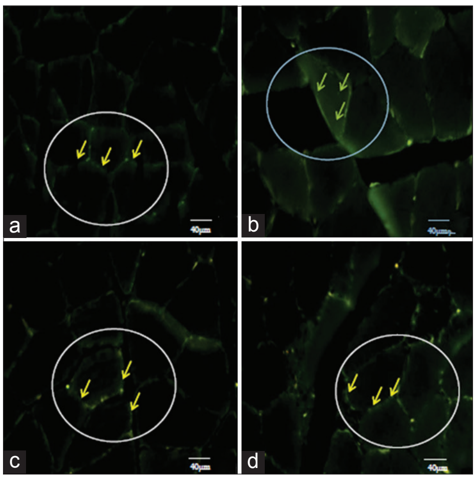

Figure 2: Photomicrographs of the immunofluorescence of skeletal muscle fibers stained with GLUT4 antibody where (a) normal, (b) negative control, (c) positive control, and (d) treated groups showing GLUT4 (yellow arrow indicated GLUT4) at Scale bar = 40 µm

Decreased GLUT4 was observed in the non-treated diabetic rats (negative controls). When compared to the non-diabetic rats (normal group), there was relatively more GLUT4 detected in the myocytes cytoplasm of the non-treated diabetic rats than at the plasma membrane as compared to the non-diabetic rats. Furthermore, in the diabetic rats treated with *O. stamineus* Benth. aqueous leaf extract, more GLUT4 was detected at the plasma membrane. This suggested that the aqueous leaf extract of *O. stamineus* Benth. has the positive effects on the translocation activity of GLUT4. Furthermore, our findings also showed statistically significant decrease in mean fasting blood glucose ($P < 0.01$) in diabetic rats treated with metformin (7.29 ± 0.30) and diabetic rat treated with aqueous leaf extract of *O. stamineus* Benth. (9.35 ± 0.72). In the non-treated diabetic rat (negative control), the fasting blood glucose level remained high (16.5 ± 1.76). The finding with *O. stamineus* Benth. was similar to the one observed by Sriplang *et al*. which showed a significant reduction in plasma glucose concentration of diabetic rats treated with the aqueous leaf extract of *O. stamineus* Benth.[15]

Table 1: Relative pancreas weight among various groups

Group	Relative pancreas weight*
Normal	6.17±1.34
Positive	4.78±1.41
Negative	3.60±0.18
Treatment	6.63±0.68

*Data are presented in Mean±SEM. SEM: Standard error of the mean

Table 2: Serum insulin concentration among various groups

Group	Serum insulin concentration*	
	Day 0 (before treatment)	Day 14 (after treatment)
Normal	1.66±0.08	1.78±0.06
Positive	ND	ND
Negative	1.86±0.03	1.76±0.09
Treatment	1.39±0.08	1.69±0.26

*Data are presented in Mean±SEM. SEM: Standard error of the mean

No significant different was observed in the relative pancreas weight and serum insulin concentration among the groups. These suggest that the improvement in glucose level is due to the GLUT4 translocation activity. This finding corroborates the findings made by Mohamed *et al*., where a reduction in blood glucose level was observed with fraction of *O. Stamineus* Benth. chloroform extract with no stimulatory effect on insulin secretion.[9]

CONCLUSION

The aqueous leaf extract of *O. stamineus* Benth. causes reduction in fasting blood glucose concentration in induced type II diabetic rats through stimulation of GLUT4 translocation to the plasma membrane without an increase relative pancreas weight and serum insulin concentration. This suggests that *O. stamineus* Benth. can be further exploited for possible treatment of insulin resistance in Type II diabetes mellitus.

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