Palestinian Herbal Plant Increases glucose disposal by skeletal muscle cell line

Hilal Zaid[1], Omar Said [1,2] and Bashar Saad [1,3]

[1] Qasemi Research Center- Al-Qasemi Academic College, P.O Box 124, Baqa El-Gharbia 30100, Israel.
[2] Antaki Center for Herbal Medicine, P.O Box 2205, Kufur kana 16930, Israel
[3] Faculty of Arts and Sciences, Arab American University Jenin, P.O Box 240, Jenin, Palestine

Introduction

Diabetes has been recognized since ancient times, and its main symptoms were known by the increased thirst, frequent urination, and tiredness experienced by diabetics (Said et al., 2008). Two main types of diabetes are known: type I, an auto-immune disease results in extensive destruction of the insulin-producing β cells, and type II also known as diabetes mellitus, leads to high blood glucose levels due to defects in insulin action (known as Insulin Resistance), excessive hepatic glucose production and eventually decreases insulin secretion. There are several types of glucose-lowering drugs (Modi, 2007), including insulin sensitizers (biguanides, metformin, thiazolidinediones), insulin secretagogues (sulfonylureas, meglitinides) and α-glucosidase inhibitors (miglitol, acarbose). Most glucose-lowering drugs, however, may have side effects, such as severe hypoglycemia, idiosyncratic liver cell injury, lactic acidosis, permanent neurological deficit, digestive discomfort, headache and dizziness (Neustadt and Pieczenik, 2008). Hence, it is crucial to search for new drugs that would potentially have no or less side effects.

Hypoglycemic herbs are widely used as non-prescription treatment for diabetes (mainly type II) (Saad et al., 2005; Saad et al., 2008; Said et al., 2008). For instance, Palestinian herbs have been used for hundreds of years either in their crude forms or as herbal teas, syrups, and powders in treatment and prevention of diverse diseases including diabetes. However, Palestinian anti-diabetic herbal-based preparations are not well characterized and there efficacies as well as their potential side effects are not demonstrated in systematic clinical trials as those of Western drugs. According to recent surveys carried out among practitioners of Arabic medicine in the Middle East, 26 plant species for the treatment of diabetes mellitus have been disclosed. This study was aimed at investigating safety and efficacy of one of these plants that is still in the process of patenting it; herein named HOB.

Materials and methods

In the present study we assessed safety and anti-diabetic effects of HOB leave extracts using *in vitro* test systems (human fibroblasts and skeletal muscle cells treated with increasing concentrations of plant extract).

Skeletal muscle is the largest site for glucose disposal in the mammalian body, and Glucose Transporter-4 (GLUT4) is the major glucose carrier in muscle (Zaid et al., 2008) mediating most of the glucose influx. Insulin activates signaling cascades that ultimately lead to GLUT4 translocation to the plasma membrane, where it can mediate glucose influx into the cell (Zaid et al., 2008; Zaid et al., 2009). Here, we focused on identifying the mechanism of action of HOB in curing insulin resistance (type II diabetes) in an *in-vitro* skeletal muscle cell line model by detecting the relative amount of GLUT4 on the plasma membrane (Figure 1).

Results

No sign of toxic effects were seen in cultured human fibroblasts and skeletal muscle cells treated with increasing concentrations of the plant mixture. Anti-diabetic effects were evidenced by measuring the relative amount of GLUT4 translocation to the plasma membrane in the presence and absence of insulin.

L6 muscle cells, stably expressing *myc*-tagged GLUT4, were utilized. These cells have been repeatedly shown to display insulin regulated GLUT4 traffic (Wang et al., 1998). L6 myotubes were pre-treated with increasing concentrations of HOB for 20h, followed by 3h

serum-deprivation and insulin stimulation for 20 min and determination of GLUT4 levels. HOB caused a dose dependent gain in surface GLUT4myc. Figure 2 illustrates the change in surface GLUT4myc caused by insulin over a range of HOB dosages, expressed as percent of the maximal insulin response.

Exposing L6 muscle cell cultures to high glucose (25 mM) and high insulin (100 nM) for 24h), followed by 3h in control medium, renders them refractory to subsequent acute stimulation by insulin. This experimental paradigm has also been tested. Consistent with the previous finding, 1mg/ml of HOB for 3h was able to overcome the induced Insulin insensitivity.

Collectively, our results demonstrate safety, tolerability and efficacy of a herbal Palestinian plant that seems to be safe for use and regulates glucose homeostasis.

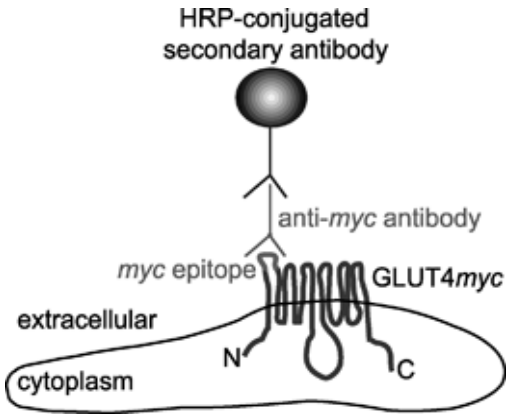

Figure1. Model demonstrating the detection of the membrane insulin-responsive glucose transporter 4 (stably expressing *myc*-tag) -GLUT4myc.

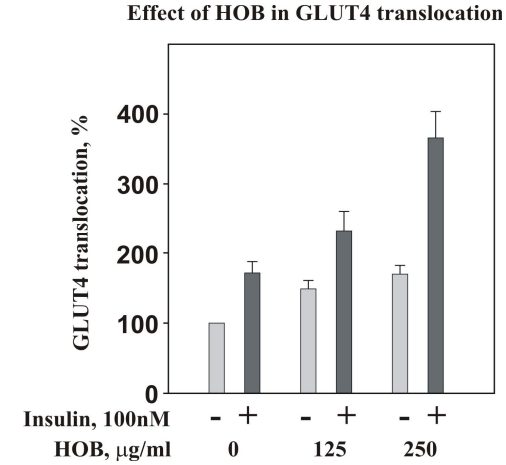

Figure2. Effect of exposure of L6-GLUT4myc myotubes to high HOB extracts in the presence or absence of insulin.

References

Modi, P. (2007). Diabetes beyond insulin: review of new drugs for treatment of diabetes mellitus. Curr Drug Discov Technol 4, 39-47.

Neustadt, J., and Pieczenik, S. R. (2008). Medication-induced mitochondrial damage and disease. Mol Nutr Food Res 52, 780-788.

Saad, B., Azaizeh, H., and Said, O. (2005). Tradition and perspectives of arab herbal medicine: a review. Evid Based Complement Alternat Med 2, 475-479.

Saad, B., Azaizeh, H., and Said, O. (2008). Arab Herbal Medicine. Botanical Medicine in Clinical Practice 4, 31.

Said, O., Fulder, S., Khalil, K., Azaizeh, H., Kassis, E., and Saad, B. (2008). Maintaining a physiological blood glucose level with 'glucolevel', a combination of four anti-diabetes plants used in the traditional arab herbal medicine. Evid Based Complement Alternat Med 5, 421-428.

Wang, Q., Khayat, Z., Kishi, K., Ebina, Y., and Klip, A. (1998). GLUT4 translocation by insulin in intact muscle cells: detection by a fast and quantitative assay. FEBS Lett 427, 193-197.

Zaid, H., Antonescu, C. N., Randhawa, V. K., and Klip, A. (2008). Insulin action on glucose transporters through molecular switches, tracks and tethers. Biochem J 413, 201-215.

Zaid, H., Talior-Volodarsky, I., Antonescu, C., Liu, Z., and Klip, A. (2009). GAPDH binds GLUT4 reciprocally to hexokinase-II and regulates glucose transport activity. Biochem J 419, 475-484.