Investigating the Synergistic Effect of Fasting and Traditional Herbs on Glucose Uptake and Insulin Sensitivity

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ABSTRACT

Introduction: Type II Diabetes Mellitus is a chronic, progressive metabolic disorder, accounting for more than 90% of diabetes cases. Current data suggest that more than half a billion adults worldwide live with T2DM and that prevalence is expected to continue rising over the forthcoming decades. The major contributors to the burden of disease include obesity, physical inactivity, and poor dietary patterns. T2DM contributes annually to millions of deaths worldwide, making T2DM one of the major contemporary global health concerns. T2DM is characterized by insulin resistance and impaired insulin secretion that can lead to hyperglycemia. Clinical chronic hyperglycemia may further result in a spectrum of complications pertaining to neuropathy, nephropathy, retinopathy, and cardiovascular disease. Classic T2DM treatments, including oral hypoglycemic agents and insulin therapy, are primarily symptomatic treatments rather than efforts at addressing any underpinning metabolic defect. Thus, many researchers embrace holistic and natural alternative therapies, including herbal interventions and intermittent fasting, which may produce benefits related to reductions in insulin resistance, improvement in glucose metabolism, and general metabolic recovery, thereby possibly being protective against metabolic defects attributed to T2DM.

Objectives: This study aims to investigate the potential role of certain traditional herbs in enhancing glucose regulation and insulin sensitivity through both in vitro (primary cell culture) and in vivo (mice model) approaches. Our primary objective, however, is to examine the efficacy of these herbal extracts in promoting glucose uptake and insulin responsiveness. Further, this study also focuses on monitoring the effects of these herbs on glucose uptake under varying glucose concentrations (normal concentration, fasting condition, and high concentration).

Methodology: Our current focus is on Phase I, which consists of in vitro experiments conducted on primary fibroblast cell cultures derived from mice kidney, mice liver, and 12-14 day old chick embryos. Fibroblasts were isolated under sterile conditions and maintained in suitable growth media. Three types of herbal extracts (methanolic, aqueous, and hydromethanolic) were prepared from eight traditional medicinal herbs, including:

1. مکی اذخر اذخر (Camel's Hay, *Cymbopogon jwarancusa*)
2. بہروزہ (Sweet Flag, *Acorus calamus*)
3. بابچی (Bakuchi, *Psoralea corylifolia*)
4. مالکنگنی (Jyotishmati, *Celastrus paniculatus*)
5. مجیٹھ (Indian Madder, *Rubia cordifoli*
6. اوٹنگن تخم (Caper Seeds, *Capparis spinosa*)
7. ناخونہ (Costus, *Saussurea costus*)
8. الطیب سنبل (Valerian, *Valeriana officinalis*)

The experiment has been designed with six different sets of treatments developed to measure glucose uptake and insulin sensitivity under varying conditions of glucose and treatment:

1. Control (Baseline): Medium (+), Glucose (–), Herbs (–), Insulin (–)
2. Glucose Challenge: Medium (+), Glucose (+) [varying concentrations], Herbs (–), Insulin (–)
3. Herbal Treatment: Medium (+), Glucose (+) [varying concentrations], Herbs (+) [varying concentrations], Insulin (–)
4. Herbs + Insulin Combination: Medium (+), Glucose (+) [varying concentrations], Herbs (+) [varying concentrations], Insulin (+) [varying concentrations]
5. Insulin Only: Medium (+), Glucose (+) [varying concentrations], Herbs (–), Insulin (+) [varying concentrations]
6. Positive Control (Metformin): Medium (+), Glucose (+) [varying concentrations], Herbs (–), Insulin (+), Metformin (+)

Following incubation, glucose uptake will be assayed using Glucose PAP-SL enzymatic assay relying on the glucose oxidase-peroxidase or GOD-PAP principle, which enables colorimetric determination of residual glucose in the culture medium. Absorbance will be measured via a spectrophotometer to estimate glucose utilization by the cells. Statistical analysis will be performed using factorial ANOVA with Tukey post-hoc testing, and $p < 0.05$ will be considered statistically significant.

Phase II studies will be based on the findings from Phase I. The most effective herbal extracts will be incorporated into the diet of diabetic mice. Phase II will evaluate the physiological impact of these extracts on blood glucose, body weight, and insulin sensitivity. This will further validate their therapeutic role in T2DM.

Key Findings: The current in vitro study reveals that the herbal extracts used significantly increase glucose uptake in the primary fibroblast cultures compared to the control setup. Methanolic and hydromethanolic extracts show more bioactivity, probably due to better solubilization of active phytochemicals. A combined treatment of herbal extracts and insulin produced a synergistic action, increasing glucose utilization both under normoglycemic and hyperglycemic conditions. Metformin was used as a positive control for comparison. Data interpretation suggests that along with the facilitation of glucose uptake, some extracts may also increase insulin sensitivity, thereby strengthening their positions as natural antidiabetic agents.

Conclusion: This study scientifically justifies the use of traditional herbal extracts as adjuvant therapeutic agents in the management of Type II Diabetes Mellitus. The in vitro findings contribute to the understanding of the role that active constituents from botanicals play in glucose regulation and their potential interactions in synergy with insulin. These results lay the groundwork for in vivo validation in diet-induced diabetic mice, furthering efforts toward developing treatment strategies that are both natural and plant-derived and thus offering scientific validation for the use of traditional medicine in the context of T2DM.

Keywords: Type II Diabetes Mellitus, Insulin Sensitivity, Herbal Extracts, Primary Cell Culture, Glucose PAP-SL Assay.

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