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Research Article

EFFECTOF BENFOTIAMINEAND SILIBININON POSTPRANDIAL HYPERGLYCEMIA OF HEALTHY SUBJECTS CHALLENGEDWITHSUCROSE LOAD: COMPARATIVE STUDYWITH ACARBOSE

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ABSTRACT

Objective: To evaluate the acute effect of single doses of benfotiamine and silibinin on postprandial hyperglycaemia after sucrose challenge in healthy subjects.

Methods: Thirteen normal healthy subjects with normal glucose tolerance were enrolled in an open label, crossover study. Theyreceived5 types of treatments separated by 72 hrs washing period. After overnight fasting, either placebo, 50mg acarbose, 100mg acarbose, 300mg benfotiamine or 200mg silibinin were administered, followed (after 60min) by oral sucrose loading (1g/kg dissolved in 150ml of water), then blood glucose levels were measured at 0, 15, 30, 60, 90, 120, 180 min postprandialy. The results of the tested compounds were compared with the standard drug acarbose and control.

Results: Pre-treatment with single doses of benfotiamine (300mg) and silibinin (200mg) results in blood glucose levels comparable to that produced by placebo and 50mg acarbose and higher than that produced by 100 mg acarbose 30 min after sucrose load. Acarbose 100mg only significantly decreases the $AUC_{0\text{-}180}$ of glucose excursions after sucrose load, while not all other types of treatments showed significant results compared to control.

Conclusion: Single doses of benfotiamine and silibinin do not reduce postprandial hyperglycemia after sucrose load in healthy subjects.

Keywords: Benfotiamine, silibinin, Postprandial glycaemia, Sucrose challenge

INTRODUCTION

There has been increasing evidence that the postprandial state is an important contributing factor to the development of many complications, including atherosclerosis and other endothelium-related vascular changes [1,2].High concentration of blood glucose as well as high glucose fluctuation during postprandial period correlates with the increase in reactive oxygen species or oxidative stress [3], which triggers with time atherogenic changes, including increases in low-density lipoprotein oxidation, sympathetic tone, vasoconstriction, and thrombogenicity [4]. Accordingly, glucose control is an important goal to diminish the risk of long-term health complications of type 2 diabetes. In addition to glycated hemoglobin and fasting plasma glucose, postprandial glucose is recently recommended as essential target for diabetes management [5].In addition to the use of insulin and many other hypoglycemic agents for controlling extended postprandial hyperglycemia and its fluctuations [6], many supplements with potential antioxidant properties are used for the same purpose [7,8].Benfotiamine, a commonly used supplement in the treatment of diabetic neuropathy [9], is a transketolase activator that directs glucose substrates to the pentose phosphate pathway; itprevents experimental diabetic retinopathy [10] and in vitro hyperglycemia-induced endothelial dysfunction [11]. Silibinin is the major compound of the silymarin isolated from seeds of Mediterranean milk thistle, *Silybummarianum* (L.) Gaertn. (Asteraceae), and clinically used as a hepatoprotectant [12].Silibinin was effective in stabilizing the mitochondrial membrane, enhancing oxidation and glycogenolysis [13];it can also rescue beta-cell function in alloxan-treated rats [14].In addition to the use of insulin and many other hypoglycemic agents for controlling extended postprandial hyperglycemia and its fluctuations, many supplements with potential antioxidant properties are used for the same purpose. Many of these agents, especially some polyphenols, are tried for their capacity to inhibit α-glucosidase activity and dampen postprandial hyperglycemia [15]. Such type of activity was mostly achieved with long-term and regular consumption of pharmacological doses of these agents [16,17]; however, the acute effect of single doses is not well established. Accordingly, the present study was designed to evaluate the effects of single oral doses of benfotiamine and silibinin dihemisuccinate on postprandial glucose spikes after oral sucrose load in healthy subjects.

METHODS

Thirteen normal healthy subjects with age range of 23-40 years and normal glucose tolerance were enrolled in an open label, crossover study. The Ethical Committee of the University of Sulaimani, Faculty of Medical Sciences, School of Pharmacy, in accordance with Helsinki declaration, approved the study protocol; the volunteers have signed an informed consent after a full explanation of the study protocol before enrollment. The volunteers were neither smokers nor alcohol drinker, and they asked to stop taking any medications especially those contain vitamins or dietary supplements, tea, coffee or any caffeine-containing drinks for at least three days before starting the test.Before starting the study, the eligibility of the volunteers have been screened by evaluating their 12 hr fasting blood glucose and only those who had FBG less than 90mg/dl were enrolled in the study; their body weight, height, medical and drug history have also been checked. Pure powders of both benfotiamine (Sigma, USA) and silibinin dihemisuccinate (Tolbiac Co, Argentina) were used in the study, and formulated as capsule dosage form containing either 300mg benfotiamine or 200mg silibinin. Blood samples for glucose level measurement were obtained by finger prick, using lancing device of an electrochemical biosensor technology Xitux Diagnostic GLAB Self-Monitoring Blood Glucose System (HMM Diagnostics GmbH-Germany). The subjects were enrolled, through a crossover design, in 5 types of treatments separated by 72 hrs washing period. After overnight fasting, either placebo, 50mg acarbose, 100mg acarbose, 300mg benfotiamine or 200mg silibinin were administered, followed (after 60min) by oral sucrose loading (1g/kg dissolved in 150ml of water), then blood glucose levels were measured at 0, 15, 30, 60, 90, 120, 180 min postprandialy. The results of the tested drugs were compared with the standard drug acarbose and control.

Statistical Analysis

The data were expressed as mean±SD. The change in plasma glucose level with respect to baseline and time was estimated to represent area under the curve. Statistical significance was performed by one

way factorial analysis of variance (ANOVA), followed by Benferroni's*post hoc* comparisons to compare means of AUC, using graph-pad prism 5 for windows software. *P* values less than 0.05 were consideredstatistically significant.

RESULTS

Oral administration of sucrose (1.0g/kg) increases blood glucose level in healthy subjects (control group) and maximum concentration (C_{max}) of glucose (140mg/dl) was achieved within 30 min (T_{max}) after ingestion of sucrose, while pretreatment with two single doses of acarbose (50 and 100mg) resulted in C_{max} of 103 and 98mg/dl respectively, 30 min post sucrose load (Figure 1).Meanwhile, pre-treatment with single doses of benfotiamine (300mg) and silibinin (200mg) results in C_{max} of 140 and 137mg/dl respectively, 30 min after sucrose load (Figure 1). In figure 2, the two doses of acarbose (50 and 100mg) significantly decrease blood glucose levels at T_{max} (30 min), compared with control, benfotiamine and silibinin groups and the effect of acarbose was dose-dependent, where the 100mg dose decreases blood glucose significantly, compared to 50mg dose at this time point (*P*<0.05).Moreover, both benfotiamine and silibinin did not decrease blood glucose levels significantly at that time point (*P*>0.05), compared with control group. In figure 3, estimation of integrated $AUC_{0\text{-}180}$ over 3 hr period revealed that acarbose 100mg only produced significant decrease in the AUC0-180 of glucose excursions after sucrose load, while all other types of treatments (50mg acarbose, 300mg benfotiamine and 200mg silibinin) did not show significant results compared to control (Figure 3).

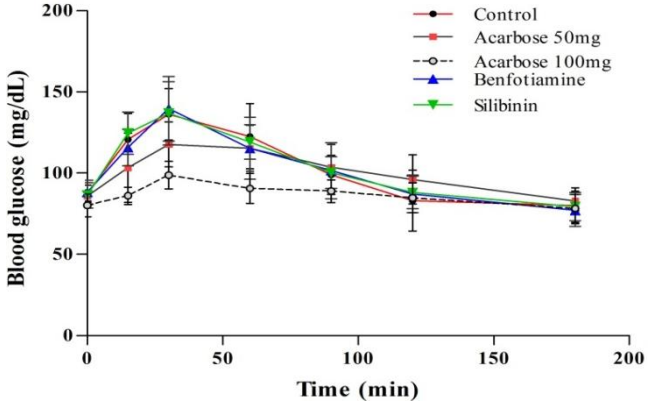

Fig. 1: Effects of single doses of benfotiamine, silibinin and acarbose on blood glucose excursions during 180 min after sucrose load in healthy subjects; *n*=13 subjects.

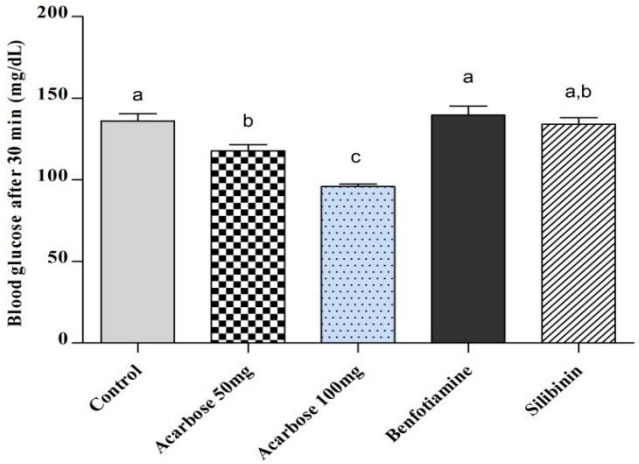

Fig. 2: Effects of single dose of benfotiamine, silibinin and acarbose on blood glucose levels 30 min after sucrose load in normal healthy subjects; *n*= 13 subjects; values with different letters (a,b,c) are considered significantly different (*P*<0.05).

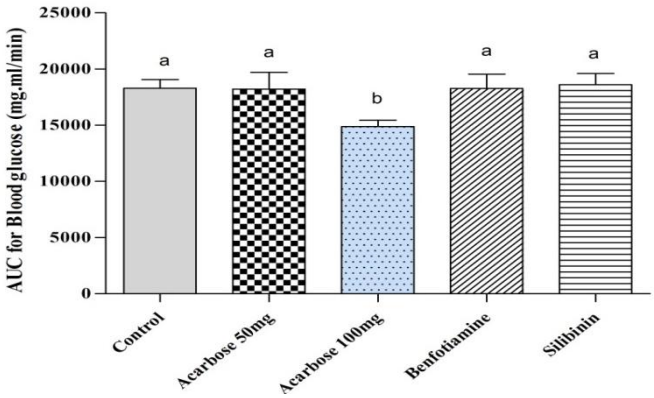

Fig. 3: Effects of single doses of benfotiamine, silibinin and acarbose on the area under the curve ($AUC_{0\text{-}180}$) of glucose levels after sucrose challenge in healthy subjects; *n*= 13 subjects; values with different letters (a,b) are considered significantly different (*P*<0.05).

DISCUSSION

Rapid absorption of glucose challenges the regulatory mechanisms of glucose homeostasis, and habitual consumption of high glycemic diets may therefore increase the risk of diabetic complications and cardiovascular diseases [18].Diet rich in carbohydrates produces sharp rise in the blood glucose level, as the complex carbohydrates in the food are rapidly digested, and absorbed as glucose in the intestine aided by the effect of the digestive enzymes α-amylase and α-glucosidase, which break dietary carbohydrates into absorbable glucose [19].Actually, controlling postprandial blood glucose level is critical in the early treatment of DM and in reducing chronic vascular complications. Therefore, interference with the processes of digestingpolysaccharides would reduce the rate of glucose release and absorption, and consequently suppress postprandial hyperglycemia [20]. Although benfotiamine is known to be useful in the treatment of certain DM complications, including retinopathy and neuropathy [10,21], and mostly long-term administration is necessary for this purpose; it fails, when used as a single dose, to lower glucose spikes in healthy subjects after sucrose load as shown in the present study. This may be attributed to its unique mechanism of action as inhibitor of aldose reductase and stimulation transketolase, which seems to be time dependent [22,23].The use of alpha glucosidase inhibitor treatment in controlof rise in the postprandial glucose level is desirable as it represents a simple effective mechanism for controlling hyperglycemia. In 2004, Abesundaraet al. reported the advantage of some plant extracts as alpha glucosidase inhibitors inprevention of progress of potential diabetic patients into T2DM patients in addition to controlling the risk ofcardiovascular damage [24].T2DM patients suffer from hyperglycemic spike up after meals, which take almost4-5 hours to reduce back to the original glucose level. Theshoot up is attributed to increased disaccharidases activityby 1.5-fold [25] and glucose transporters GLUT2 and SGLT1activity by almost 3-4-fold in diabetic animals as comparedto normal [26]. The increased glucose level for prolongedtime leads to nonspecific glycation of proteins initiating acascade of secondary complications [27]. Hence, control ofpostprandial glucose levels would be valuable in preventionof secondary complications of diabetes.Previous research has been focused on the control of postprandial glucose by the inhibition of pancreaticamylaseand the intestinal-glucosidases, the key enzymes of dietary carbohydrate digestion [28].Slowing the rate of carbohydrate digestion and/or absorption is the most probable mechanism underlyingthe potential for attenuated postprandial hyperglycemia, as this condition is associated with the preventionof impaired glucose tolerance (pre-diabetes) and a significant reduction in risk of developingT2DM [29].It has been reported previously that the treatment with an α-glucosidase inhibitor(acarbose) specifically delays postprandial hyperglycemia, reduced the risk of T2DM [30].It ispossible that consumption of certain polyphenols, including silibinin, will prevent or delay developing T2DM in healthy people.An evidence reports that suppression of postprandial glucose may contribute to decreasing thelevel of HbA1c resulting in a significant reduction in the incidence of chronic vascular complication such as macro- and micro vascular diseases [31].In the present study, we examined the acute effect of silibinin on the postprandial effects of oral sucrose in healthy subjects. We report that treatment with silibinin(200mg) do not reduce the postprandial elevation in blood glucose levels induced by bolus dosingwith sucrose; this result was in tune with that reported byLambert et al., where single acute dose of green tea polyphenols produced no effect on the increase in postprandial blood glucose induced by maltose orglucose [32].Therefore, these results suggest that silibinin may modulate an aspect of carbohydrate metabolism that is unique to polysaccharides, which might be the result of inhibition of α-amylase by silibinin. However, although the present study includes only healthysubjects, it may nevertheless apply to individuals with diabetes, as hyperglycemia is an independentpredictor of future cardiovascular events in both healthy and diabetic individuals. Therefore, we assume that an intakeof silibinin might help people with T2DMto control the postprandial hyperglycemia asthereby prevent the progression of diabetic complications.The recently published data showed that silibinin (200mg/day) reducesfasting plasmaglucose, postprandial hyperglycemia and HbA1c level in diabetic patients after four months of supplementation[16]. However, theeffect of single acute dose of silibinin on postprandial glucose level in healthy subjects remains controversial.Further studiesare needed to determine the acute effect of silibinin in diabetic subjects focusing onexamining postprandial glucose and HbA1c level, which could yield important new insights into thepreventive of diabetic complications. Another recent reports suggested that a lifetime intake of dietarypolyphenols, assuming the same mechanism, has a comparable potential to reduce diabetes risk [33], but more studies are required to fully test the effect of modulating post-prandial blood glucose in healthy subjects and those with DM. In conclusion, single acute doses of benfotiamine and silibinin do not affect postprandial glucose excursions after sucrose load in healthy subjects.

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