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Original Article

Genistein Improves Blood Sugar and Glycated Hemoglobin Levels in Diabetic Rats

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ABSTRACT *Background :* **Diabetes mellitus is associated with increased risk of cardiovascular disease and other serious complications. Genistein, the active ingredient of soy product, has been suggested for its antioxidant and its endothelial functional improvement. However, the information of genistein on diabetes is presently limited.**

Aim To study the response of glucose and lipid in genistein treated diabetic rats.

Material and Methods : **Male Wistar rats weighing 180-200 g were divided randomly into two major groups of diabetes (DM) and non-diabetes (CON). STZ-induced rats were used in the present study. In diabetic group, four subgroups were further randomly divided as follows: 1.) Diabetic groups received 100 µl of dimethylsulfoxide for 4 and 8 weeks (4-wk DM+DMSO and 8-wk DM+DMSO). 2) Diabetic groups received daily injection of 0.25 mg /kg bw genistein for 4 and 8 weeks (4-wk DM+Gen and 8-wk DM+Gen). The non-diabetic controls were housed for 4 and 8 weeks before termination. Fasting blood glucose, hemoglobin A1C, total cholesterol, triglycerides, HDL-C, and LDL-C were compared at the end of 4 and 8 weeks.**

Results : **Blood glucose levels were significantly decreased at both 4 (DM+DMSO = 346.16 ± 18.39 mg/dL, DM+Gen = 276.50 ± 20.01 mg/dL; $p < 0.05$) and 8 weeks (DM+DMSO = 465.83 ± 32.72 mg/dL, DM+Gen = 165.66 ± 25.46 mg/dL; $p < 0.05$) of genistein treatment, whereas HbA_{1c} level was significantly attenuated at 8 weeks (DM+DMSO = 10.08 ± 0.45 %, DM+Gen = 7.71 ± 0.40 %; $p < 0.05$). However, genistein did not have any effects on plasma lipid profiles.**

Conclusion : **Genistein has a hypoglycemic property without lipid lowering effect. Therefore, genistein may be beneficial to diabetic control and help reducing its complications.**

INTRODUCTION

Diabetes mellitus is characterized by several metabolic impairments. Alteration in carbohydrate and fat metabolism in diabetes is closely related to hyperglycemia. Abnormal lipid and lipoproteins metabolism, high concentration of triglyceride-lipoprotein in particular, is common in poor glycemic control[1,2].

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Phytoestrogens are naturally occurring plant based diphenolic compounds that are similar in structure and function to estradiol. Although there are many types of phytoestrogens, the most common and well-studied phytoestrogen is the class of isoflavones. The abundant active components of isoflavones are genistein and daidzein. While these agents appear to have selective estrogenic actions, the recent identification of a second subtype of estrogen receptor lends support to the theory of selective estrogenic properties[3,4]. It has been reported that genistein binds estrogen receptors with 100 times less affinity compared to estrogen[5]. Therefore, biological effects may occur as enough quantity can be con-

sumed from the isoflavones enriched diet.

It has been postulated that soy isoflavones, structurally similar to estrogens, interact with estrogen receptors and may decrease serum cholesterol concentrations by similar mechanisms[6]. There is evidence that soy protein reduced LDL-C by 13 percent, lowered plasma triglycerides by 10 percent, and possibly increased HDL-C by 2 percent[7]. In contrast, several studies have reported unclear effects of phytoestrogen on lipid profile. Lipid levels did not change with isoflavone supplement in hypercholesterolemic postmenopausal women[8].

Although some evidence has suggested that phytoestrogen may exert a positive role on lipid profiles, metabolic effects of phytoestrogens have not yet been well clarified in diabetes. Therefore, the objective of the present study was to study the effect of genistein on lipid profiles, plasma glucose, and HbA_{1C} in diabetic rats.

MATERIAL AND METHOD

Animal preparation

Male Wistar rats (National Laboratory Animal Center of Salaya Campus, Mahidol University, Thailand) weighing 180-200 g, 6-weeks old were divided randomly into three groups:

Control group (Control) (n=12): normal rats were received a single intravenous injection of citrate buffer (65 mg/kg bw). The animals were supplemented with normal saline 100 µl/day injected subcutaneously.

Diabetic group (DM+DMSO) (n=12): diabetic was induced tube diabetes by a single intravenous injection of streptozotocin (STZ; 65 mg/kg bw). After 48 hours of STZ injection the animals were daily supplemented with dimethyl sulfoxide (DMSO; Sigma Chemical Co., USA) 100 µl/day injected subcutaneously.

Diabetic genistein-treated group (DM+Gen) (n=12): rats were induced the same way as described for the diabetic group. Forty-eight hours after administration of STZ, the animals were daily supplemented with genistein 0.25 mg/kg bw in 100 µl of DMSO/day (Sigma Chemical Co., USA) injected subcutaneously.

All animals were fed with regular dry rat chow and allowed freely access to drinking water. In this study each group was further divided into 2 subgroups according to the time of collection of the specimen i.e, at 4 weeks (n=6) and 8 weeks (n=6).

Diabetic induction

To induce diabetes mellitus, STZ (Sigma Chemical Co., USA) was freshly prepared by dissolving in citrate buffer (Sigma Chemical Co., USA) pH 4.5 and immediately single injected into the tail vein of 8-hours fasted rats, at a dose of 65 mg/kg bw. To confirm the success of diabetic induction, blood glucose was determined by using glucometer (Advance Glucometer, Bochringer Mannheim, Germany). Samples were analyzed by applying a drop of blood to a prepared strip. Rats treated with STZ that did not exhibit an elevation of blood glucose level greater than 200 mg/dL[9] at 48 hours were excluded from the study. In addition, diabetic condition was also confirmed by manifestations of polyuria, polyphagia, and polydipsia of the animals.

Blood sampling

Blood samples were collected at the end of the experiment. Plasma was separated from whole blood by centrifugation with 3,500 rpm at 4 ˚C for 15 minutes, then it was stored at a temperature of 4 ˚C until being analyzed at the BRiA LAB CO., LTD. The analysis of total cholesterol, triglyceride, HDL-C, LDL-C, HbA1C and glucose in the blood was determined by using an automatic analysis technique on a chemical analyzer form Roche diagnostics (COBAS INTEGRA 700) as follows.

Plasma total cholesterol, triglycerides, HDL-C, LDL-C

Cholesterol is determined by enzymatic, colorimetric method (CHOD/PAP) with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine. Triglyceride levels is determined by enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and and 4-aminophenazone. HDL-C concentration is determined after isolation of HDL-C in the specimen. The separating reagent for HDL-C uses phosphotungstic acid and magnesium ions to precipitate the chylomicrons, VLDL, and LDL. After centrifugation, HDL remaining in the supernatant is quantitated by its cholesterol content. The HDL-C concentration is determined using an enzymatic, colorimetric method (CHOD/PAP). LDL-C concentration is determined using an enzymatic, colorimetric method (CHOD/PAP).

HbA_{1c} and blood glucose levels

Total Hb and HbA_{1c} concentrations are determined after hemolysis of the anticoagulated whole blood specimen. Total Hb is measured colormetrically. HbA_{1c} is determined immunoturbicimetrically. The ratio of both concentration yields the final percent HbA_{1c} result [HbA_{1c} (%)]. Blood glucose is determined using enzymatic reference method with hexokinase.

Data analysis

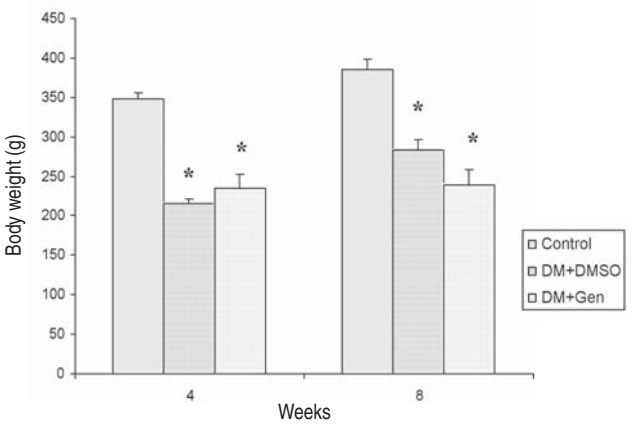
The results are expressed as means ± SEM. Statistical analysis of differences between groups at each time point was performed using one way ANOVA followed by post hoc Bonferroni's test. Probability values of less than 0.05 were considered statistically significant.

RESULTS

Rats with intravenous injection of STZ 65 mg/kg bw significantly developed hyperglycemia within 48 hours and showed persistent hyperglycemia throughout the experiment.

Body weight

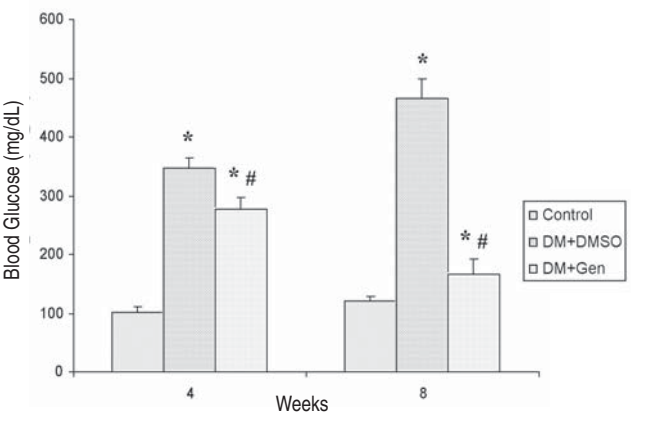
In order to determine the effect of gross metabolic effect of diabetes, the changes in body weight (g) were determined. The DM+DMSO and DM+Gen groups had significantly decreased body weight compared to the control group at 4 and 8 weeks. The DM+Gen group had no significant difference compared to the DM+DMSO group at 4 and 8 weeks. (Figure 1)



Values are means ± SEM; n = 6

* Significant difference as compared to control ($p < 0.05$).

Figure 1 Body weight (g) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.



Values are means ± SEM; n = 6

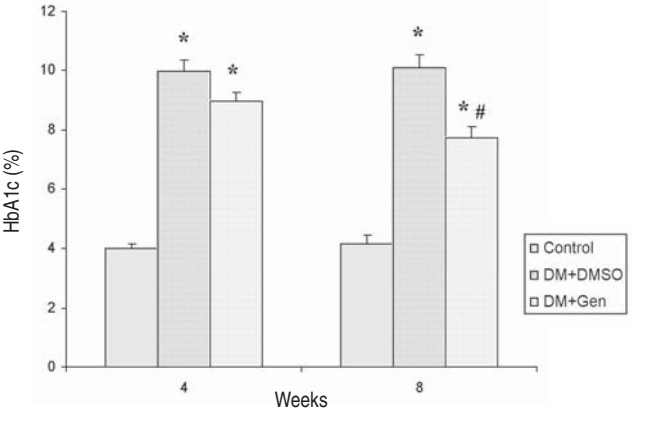
*Significant difference as compared to control ($p < 0.05$).

#Significant difference as compared to DM+DMSO ($p < 0.05$).

Figure 2 Blood glucose (mg/dl) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.

Blood Glucose and HbA_{1c}

The results revealed that the DM+DMSO and DM+Gen groups had significantly increased blood glucose and HbA_{1c} compared to the control group. At 4 and 8 weeks, the DM+Gen group had significantly decreased blood glucose compared to DM+DMSO group (Figure 2). There was a trend for a further decreased



Values are means ± SEM; n = 6

*Significant difference as compared to control ($p < 0.05$).

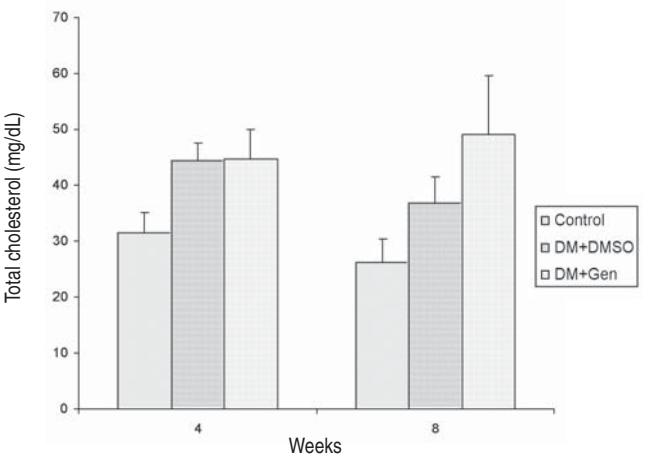
#Significant difference as compared to DM+DMSO ($p < 0.05$).

Figure 3 HbA1c (%) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.

blood glucose with genistein treatment (276 ± 20 vs 165 ± 25 mg/dL, for weeks 4 vs weeks 8), while the diabetic rats without genistein showed a worsening blood glucose level (346 ± 18 vs 465 ± 33 mg/dL, for weeks 4 vs weeks 8). At 4 weeks, HbA1c in the DM+Gen group (8.95 ± 0.33 %) had no significant difference compared to the DM+DMSO group (9.98 ± 0.38 %) but the DM+Gen group (7.71 ± 0.4%) showed a significant lower HbA1c level compared to the DM+DMSO group (10.08 ± 0.45%) at 8 weeks. (Figure 3)

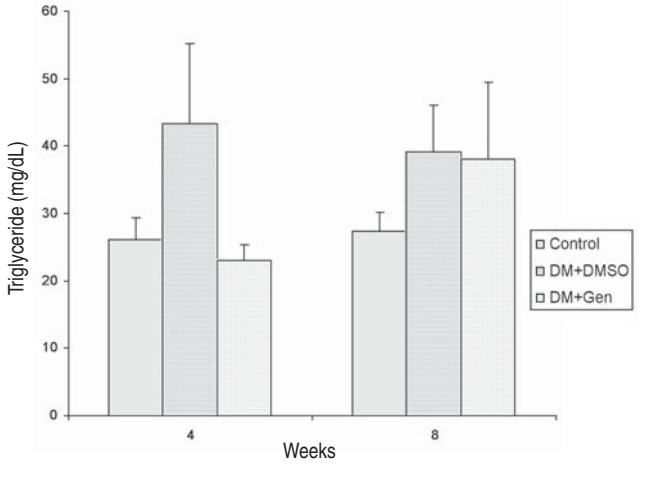
Lipid profiles (Figures 4-7)

There were no differences among the DM+DMSO, DM+Gen, and control groups on plasma total cholesterol, triglycerides, HDL-C or LDL-C at 4 weeks. Similarly, the 3 groups did not show any difference on lipid profile at 8 weeks.



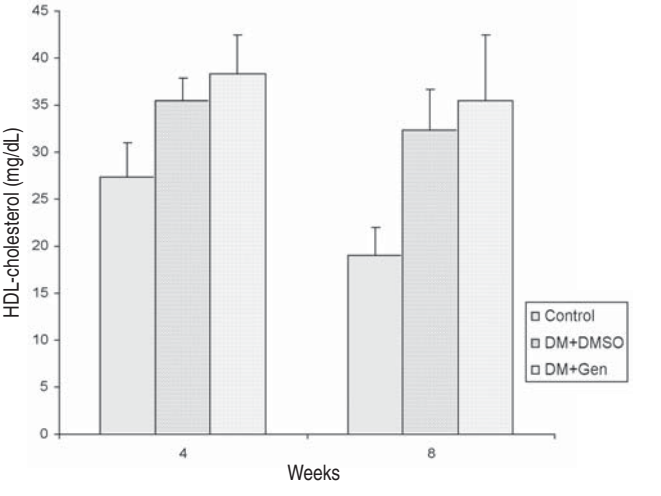
Values are means ± SEM; n = 6

Figure 4 Total cholesterol (mg/dl) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.



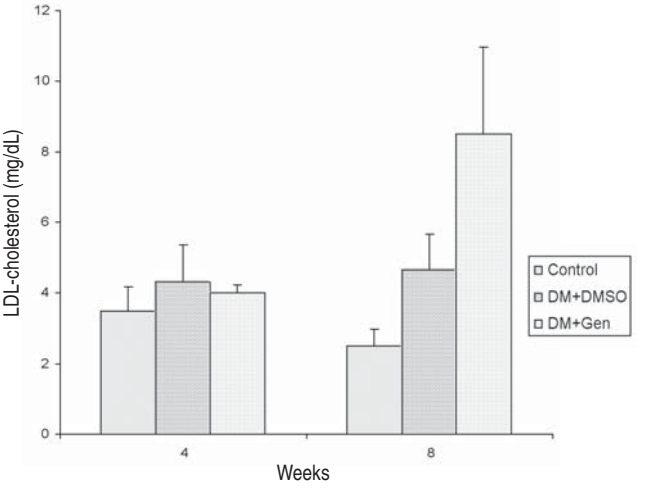
Values are means ± SEM; n = 6

Figure 5 Triglycerides (mg/dl) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.



Values are means ± SEM; n = 6

Figure 6 HDL-cholesterol (mg/dl) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.



Values are means ± SEM; n = 6

Figure 7 LDL-cholesterol (mg/dl) of control treated rats, DMSO treated rats, and genistein treated rats at 4 and 8 weeks.

DISCUSSION

The main objective of the present study was to demonstrate the effect of genistein on metabolic profiles particularly blood sugar and lipid levels in rats with STZ-induced diabetes. During the period of 4 and 8 weeks of the experiment, we found no change in lipid profile in animals treated with genistein. However, we demonstrated that genistein reduces blood glucose levels in diabetic rats as early as 4 weeks and the reducing effect on HbA_{1c} was subsequently evident in 8 weeks. As of our knowledge, we are the first to demonstrate that blood glucose and HbA_{1c} levels can be decreased with genistein treatment in a different time period in experimental diabetic rats.

It has been reported that genistein consumption significantly decreased fasting glucose in healthy postmenopausal women[10]. A recent study in animals supplemented with genistein containing diet (600 mg/kg diet) showed a reduction in HbA_{1c} levels[11]. The mechanism of genistein-induced decline in blood glucose levels was unclear. There were two earlier reports suggesting that genistein could stimulate insulin secretion from pancreatic β cells via independent protein tyrosine kinase (PTK) inhibition[12,13]. A more recent study had added more insight into data that 50 μM genistein significantly potentiated glucose-stimulated insulin release from rat islets through the voltage-dependent Ca^{2+} channel which regulated Ca^{2+} influx into β cells after food intake[14]. Unfortunately the insulin levels were not measured in the present study. However, hypoinsulinemia could be expected in our animals since the STZ model of diabetes has been well known to cause damages of pancreatic β cells. As there may have a few β cells left undestroyed, genistein may be able to stimulate the survived β-cells to secrete more insulin and exert its hypoglycemic action. Therefore, the effect of genistein on HbA_{1c} was reasonably shown at 8 weeks after treatment due to the cumulative effect on the reduced blood sugar over the course of treatment period.

Our findings showed that levels of cholesterol, triglyceride, HDL-C and LDL-C were unchanged in both 4 and 8 weeks of diabetic development. Similarly, genistein treated group demonstrated no significant difference on any of the lipid measures as compared to their age-matched DM+DMSO groups both in 4 and 8 weeks. In human, dyslipidemias are often found in individuals with diabetes. The fact that the animals in our study did not develop dyslipidemia was probably due to an inadequate duration being diabetic to develop changes on lipid profile.

During 1960 to 1993, several studies on the hypocholesterolemic effects of soy protein in both human and animal models had been performed. However, the American Heart Association (AHA) had concluded that soy protein could lower serum cholesterol only in animals but not in human[15]. Some studies suggested that 30-50 g/day of soy protein supplement was needed for LDL-cholesterol reduction[16]. It is unclear that the dose of genistein used in our study was comparable to the effective dose in the form of soy protein the in Tonstad's report. Moreover, the study in humans with dyslipidemia may not be able to interpret the same way as in diabetic animals.

The fact that we demonstrated impairment in carbohydrate metabolism in STZ-rats despite no significant change in lipid profile suggested that the abnormality in glucose metabolism in diabetes mellitus does not concurrently lead to lipid abnormalities. In addition, the effect of genistein in improving carbohydrate metabolism in diabetes did not have to be coincided with lipid improvement.

In conclusion, metabolic alterations in rats with STZ induced diabetes can be improved by daily genistein supplementation. The effects of genistein on fasting glucose and glycated hemoglobin may be potentially beneficial to the future diabetic control.

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