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Original Article

# Glycemic effect of stevioside and *Stevia rebaudiana* in streptozotocin-induced diabetic rats

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**Abstract.** The present experiment was conducted to study the glycemic effect of stevioside (SVS) and clarify whether SVS participates in glycemic action of aqueous extract of *Stevia rebaudiana* (SR) in diabetic rats. Male Wistar rats weighing between 150-180 g were used. Six groups of rats were established. The first three groups were normal rats daily fed by intragastric intubation of water or SVS (0.25 g / kg body weight) or SR at the dose corresponding to the dose of SVS administration (4.66 g / kg body weight) for 8 weeks. The other three groups were diabetic rats treated with the same procedure as normal rats. The plasma glucose level ($P_G$) was determined once a week after overnight fasted. At the end of experiment, serum insulin and plasma glucagon levels were determined. Insulin-induced glucose uptake by isolated diaphragm was also evaluated using 2-deoxy-D-[$^3$H]-glucose and [$^{14}$C]-manitol. The results show that SVS slightly but significantly raised $P_G$ since the third week in normal rats whereas no significant change was noted in diabetic rats throughout experimental period. The $P_G$ was not altered in normal rats fed with SR whereas it significantly reduced since the second week until the end of experiment in diabetic rats fed with SR. Serum insulin and plasma glucagon levels were not significantly changed in normal rats fed with either SVS or SR. Serum insulin was raised to the same extent in diabetic rats fed with either SVS or SR. The high plasma glucagon level was suppressed in diabetic rats fed with SR to the level that was not significantly different from that of the normal rats. Insulin-induced glucose uptake by isolated diaphragm muscle were suppressed in both normal and diabetic rats fed with SVS whereas no change was apparent in both normal and diabetic rats fed with SR. A suppression of insulin-induced glucose uptake by muscle plays a predominant role to produce hyperglycemia in normal rats fed with SVS. Despite an improvement of insulin level, anti-hyperglycemic effect of SVS can not be displayed in diabetic rats since a reduction of insulin-induced glucose uptake was noted as well. An elevation of insulin and suppression of glucagon are the primary cause of anti-hyperglycemic effect of SR in diabetic rats. SVS containing in SR may indirectly contribute to anti-hyperglycemic action of SR in diabetic rats via its effect to potentiate insulin release.

Keywords : Stevioside, *Stevia rebuadiana* , glucose uptake, blood glucose

## 1.Introduction

Diabetes mellitus (DM) is a metabolic disease which leads to severe organ damages and death. The incidence of DM is considered to be high all over the world. In spite of the discovery of new hypoglycemic agents, DM and its related complications continue to be a major problem. Medical plants has been recently an increasing interest to treat DM. Ethnobotanical information indicates that more than 500 plants are used as traditional remedies for DM treatment (1). *Stevia rebaudiana* (SR) is a sweet herb of South America. SR and its major component, stevioside (SVS), have been used as sugar substitute and medical treatment for centuries by the natives of Paraguay and Brazil (2). SVS was found to be a non-caloric sweetener, and it is being of increasing interest in several countries as a new non-caloric sweetener (2). Because of its great sweetness, cultivation of SR has now spread to several countries including Japan, Canada, USA, Korea, China and Thailand. Apart from its sweetness, SR and SVS have also been reported to possess various physiological effects such as cardiovascular effects (3,4), renal effects (5,6) microbiological effects (7) and endocrine effects (8). However, little data are available to support their glycemic effect since only in the form of

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abstracts have been published with little evidence. Furthermore, most experiments have been performed to elucidate their glycemic actions during euglycemic condition whereas no evidence support their action in DM. Crude extract of SR has been demonstrated to lower plasma glucose level in rats fed with high carbodydrate diet containing 10% dried SR leaves for 4 weeks (9). It has been shown that glucose tolerance was raised in normal volunteers subjected to aqueous extract of SR (10). Moreover, SVS has been recently found to induce insulin release from isolated rat pancreatic islets (11). It is possible that SR and SVS are able to reduce plasma glucose level in diabetic condition. Therefore, the present experiment was conducted to evaluate glycemic effect and the mechanism of action of both SVS and SR in streptozotocin-induced diabetic rats. The experiment was also studied to clarity whether the glycemic effect of SR is mediated by the action of SVS containing in SR.

Table 1

The effect of stevioside and aqueous extract of Stevia rebaudiana on the serum insulin and plasma glucagon concentrations in both normal and diabetic rats (control, normal rats ; DM, diabetic rats ; SVS, stevioside ;SR, stevia rebaudiana)

| Groups | Serum insulin concentration (μIU/mL) | Plasma glucagon concentration (pg/mL) |
|---|---|---|
| Control (n = 7) | 5.13 ± 0.19 | 45.89 ± 6.59 |
| Control-SVS (n = 8) | 6.19 ± 0.15 | 48.30 ± 6.62 |
| Control-SR (n = 8) | 5.17 ± 0.36 | 47.08 ± 4.95 |
| DM (n = 8) | 2.66 ± 0.19 | 76.04 ± 5.38 |
| DM-SVS (n = 8) | 3.29 ± 0.11* | 75.21 ± 3.12 |
| DM-SR (n = 9) | 3.87 ± 0.45* | 49.43 ± 3.45# |

Values are mean ± SEM *,# significant difference comparing to DM at P<0.05 and P<0.01 respectively

## 2.Material and methods

### *2.1. Animal preparation*

Male Wistar rats weighing between 150-180 g were used. All animals were cared for in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn University which is under the National Council of Thailand for animal care. Normal rats food and tap water were supplied *ad libitum*. Diabetic induction was carried out by intraperitoneal injection of streptozotocin (65 mg / kg body weight) dissolved in normal saline whereas normal rats were injected with normal saline. After 24 hours of streptozotocin injection, only rats with fasting plasma glucose level over than 250 mg/dl were included in the experiment.

### *2.2.Experimental design*

Two series of experiment was undertaken

Table 2

The changes of 2-deoxy-D-[$^3$H]-glucose uptake by diaphragm muscle isolated from normal and diabetic rats either treated with or without SVS or SR (control, normal rats ; DM, diabetic rats ; SVS, stevioside; SR, Stevia rebaudiana ; ICF $H_2O$, intracellular water)

| Groups | 2-Deoxy-D-[$^3$H]-glucose uptake (nmol/mL ICF $H_2O$) |
|---|---|
| Control | 39.37 ± 3.59 |
| Control-SVS | 24.16 ± 1.56* |
| Control-SR | 40.67 ± 3.81 |
| DM | 42.63 ± 1.66 |
| DM-SVS | 33.99 ± 2.55# a |
| DM-SR | 40.72 ± 3.11 |

Values are mean ± SEM *significant difference comparing to control at P<0.05 #significant difference comparing to DM at P<0.01 [a] significant difference comparing to control-SVS at P<0.05

### *Series I : The effect of stevioside and Stevia rebaudiana on the plasma glucose level in both normal and diabetic rats*

Six groups of seven to eight animals each were established including three groups of normal and three groups of diabetic rats. In the first three groups, normal rats were daily fed by intragastric intubation of distilled water or SVS at the dose of 0.25 g/kg body weight or aqueous extract of SR at the dose corresponding to the amount of SVS administration (4.66 g/kg body weight). Another three groups of diabetic rats were performed in the same maneuver as normal rats. SVS (approximately 95-97% purity) was prepared and purified from dried *Stevia rebaudiana* leaves as described by Adduci et al (12). All animals were daily fed with water or SVS or SR for 8 weeks.

In each week, blood was collected from tail vein to determine $P_G$ after overnight fasted. The plasma glucose concentration was determined using glucose oxidase (Glucose liquicolors, Human Co.,Ltd. Taunusstein, Germany)

*Series II : The mechanisms of glycemic action of stevioside and Stevia rebaudiana.*

There are several mechanisms controlling plasma glucose including glucose regulating hormones and tissue glucose uptake. Both mechanisms were undertaken in the present experiment.

*a.Determination of glucose regulating hormones*

After 8 weeks of SVS or SR administration, rats were overnight fasted and then anesthetized by intraperitoneal injection of sodium pentobarbiturate (50 mg /kg body weight). Blood was collected from abdominal aorta to determine serum insulin and plasma glucagon concentrations using radioimmunoassay (Diagnostic Product, Co.,Ltd, LA,USA).

*b.Determination of tissue glucose uptake.*

After 8 weeks of SVS or SR feeding, rats were fasted overnight and then anesthetized by sodium pentobarbiturate. Abdominal incision was performed to isolate diaphragm as modify method described by Kipnis and Cori (13). Isolated diaphragm was dissected and rapidly washed in Krebs-Henseleit buffer (KHB) containing 118 mmol NaCl, 4.7 mmol KCl, 2.5 mmol $CaCl_2$, 1.2. mmol $KH_2PO_4$, 1.2 mmol $MgSO_4$ and 25 mmol $NaHCO_3$, pH 7.4. The isolated muscle (approximately 100 mg each) was then incubated in the solution containing 1.5 ml KHB plus 5 mmol glucose, 10 mmol manitol, 0.1 g/ 100 ml BSA (RIA grade), 1 mmol sodium pyruvate, pH 7.4 with100 milliunits of insulin (Humulin R, Eli Lilly, Indianapolis IN) for 20 min . The muscle sample was gassed continuously with 95% $O_2$-5% $CO_2$ and maintained at the temperature of 37 $^{\circ}$C throughout experiments. After 10 min of incubation, the muscle sample was then transferred to KHB (1.5 ml) containing 5 mmol 2-Deoxy-D-[2,6 $^3$H]-glucose (2 μCi/ml) (44 Ci/mmol, Amersham, Buckinghamshire,UK), 10 mM D-[1$^{14}$C]-manitol (0.2 μCi/ml)(56 mCi/mmol Amersham, Buckinghamshire, UK) 0.1 g/ 100 ml BSA, 1 mmol sodium pyruvate in the presence of insulin. After 30 min of incubation, the experiment was terminated by quickly rinsing the muscle sample in ice cold KHB three times over a period of 1-2 min, and then blotted with filter paper and weighted. The muscles and the remaining incubation medium were frozen at -70 $^{\circ}$C until processed. The muscles were processed by homoginizing in 5% trichloroacetic acid. The homogenate was then centrifuged at 5,000 g at 0$^{\circ}$C for 10 min. Supernatant and incubation medium were counted for radioactivity by liquid scintillation spectrometer (Packard Instrument ,Zurich, Swithzerland). The amount of 2-deoxy-D-[$^3$H]) glucose (2-DG-[$^3$H]) taken up by the muscle was determined after correcting for the extracellular space using D-[$^{14}$C] mannitol (14). The intracellular 2-DG[$^3$H] glucose concentration as expressed in nmol/ml intracellular water (ICF $H_2O$) was determined.

*2.7.Statistical Analysis*

All values were shown as mean ±SEM. Statistical analysis was accomplished using student's unpaired t-test. Data were considered to show a significant difference at the level of $P<0.05$.

## 3.Results

*3.1.Effect of stevioside and Stevia rebaudiana administration on the plasma glucose level*

Fig 1 shows the effects of SVS and SR feeding on $P_G$ in both normal and diabetic rats. $P_G$ was slightly but significantly raised in normal rats fed with SVS since the third week. In contrast, SR had no significant effect on the $P_G$ in normal rats. SVS had no effect on the $P_G$ in diabetic rats (DM-SVS) whereas SR significantly reduced the $P_G$ in diabetic rats (DM-SR) since the second week till the end of experiment (Fig 2).

*3.2.The mechanisms of glycemic action of stevioside and Stevia rebaudiana*

The alteration of both serum insulin and plasma glucagon levels is shown in table 1. The serum insulin level in normal rats treated with SVS or SR was not significantly different from normal rats fed with water. The serum insulin level was raised from 2.66 ±0.19 μIU/ml in normal diabetic rats to 3.29±0.11 μIU/ml ($P<0.05$) in DM-SVS

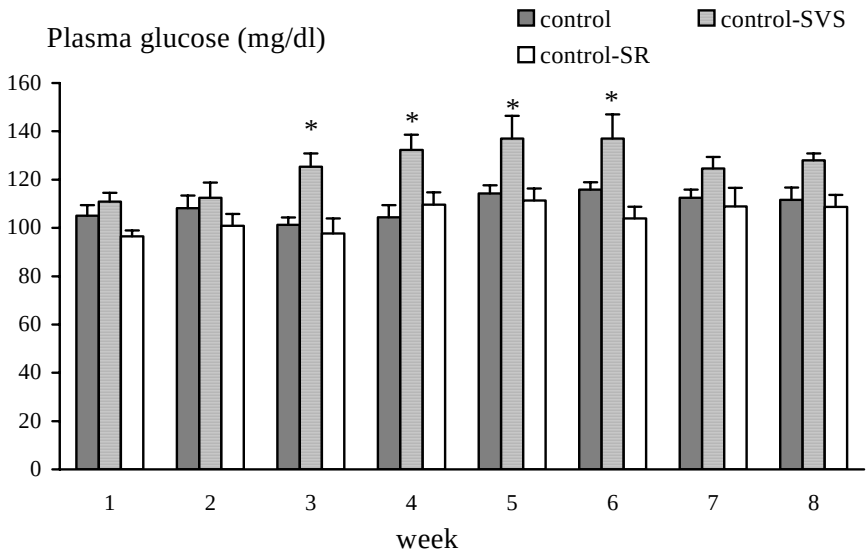


Fig.1.Changes of plasma glucose concentration in normal rats fed with water or stevioside or *Stevia rebaudiana* (control ;normal rats ; control-SVS, normal rats fed with stevioside ; control-SR, normal rats fed with aqueous extract of *Stevia rebaudiana*). Values are mean ± SEM.*significant difference comparing to control in the same week.

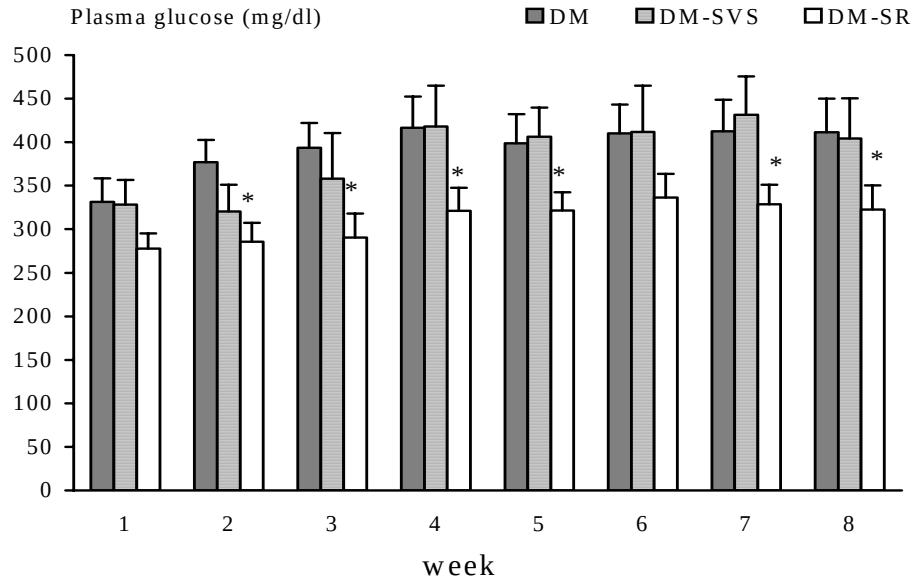


Fig. 2. Changes of plasma glucose concentration in diabetic rats treated with or without stevioside or Stevia rebaudiana (DM; diabetic rats ; DM-SVS, with stevioside ; DM-SR, diabetic rats fed with aqueous extract of Stevia rebaudiana). Values are mean ± SEM. *significant difference comparing to DM rats in the same week.

and to 3.87±0.45 μIU/ml ($P<0.05$) in DM-SR. Both SVS and SR administration had no significant effect on the plasma glucagon level in normal rats. The plasma glucagon concentration in DM-SVS was not different from that of normal diabetic rats (76.04±5.38 & 75.21±3.12 pg/ml) whereas DM-SR exhibited a depression of the plasma glucagon level to the normal value (49.43±3.45 pg/ml, $P<0.01$).

Insulin-induced glucose uptake by the isolated diaphragm muscle is shown in table 2. Glucose uptake by the isolated diaphragm muscle of normal rats fed with SVS was significantly reduced ($P<0.01$) whereas no significant change was apparent in normal rats fed with SR. Similary, glucose uptake was slightly but significantly suppressed from 42.63±1.66 in normal diabetic rats to the level of 33.99±2.55

nmol/ml ICF $H_2O$ in DM-SVS ($P<0.05$) whereas no significant change was noted in diaphragm muscle isolated from DM-SR (40.72±3.11). Though there was a depressive response of insulin-induced glucose uptake by the isolated diaphragm muscle of diabetic rats fed with SVS but the level was remained significantly higher than that of normal rats fed with SVS ($P<0.05$)

## 4.Discussion

*Stevia rebaudiana* (SR) composes of several glycosides such as stevioside (SVS), rebaudioside A,B,C,D. Both SVS and SR have been mostly used as non-caloric sweetener with little medical application. Little data are available to clarify the glycemic effect of both SVS and SR. Furthermore, most of experiments have been underwent during euglycemia. Experimental evidence to support their glycemic actions in hyperglycemic condition is lacking. Streptozotocin-induced hyperglycemia has been described as a useful experimental model of study in the activity of hypoglycemic agents. In the present study, daily feeding of SVS for 8 weeks slightly but significantly raised $P_G$ in normal rats. This is not owing to glucose contamination in SVS since there was too small amount of glucose containing in SVS (2-3 mg/100 mg SVS) to account for hyperglycemia. Although some part of SVS feeding was degraded to glucose and then absorbed through intestine, the absorbed glucose was continuously metabolized (2). Therefore, hyperglycemia in normal rats fed with SVS should be the action of SVS itself. For diabetic rats, SVS had no effect on the $P_G$ whereas SR alleviated the hyperglycemia. This implies that SR has anti-hyperglycemic action in diabetic rats, and its action is not caused by SVS containing in SR. Some glycosides containing in SR may be responsible for this activity. Up until now, no experimental study has investigated the glycemic effect of other glycosides containing in SR except SVS.

The mechanisms responsible for the glycemic effect of SVS and SR have been proposed in the present experiment. Both SR and SVS had no significant influence on serum insulin and plasma glucagon levels in normoglycemic rats whereas they improved insulin level in diabetic rats. A facilitative effect of SR on insulin level in DM-SR should be the action of SVS containing in SR since insulin level in DM-SVS and DM-SR was quite the same extent. Moreover, SVS has been recently reported to stimulate insulin release from isolated pancreatic islets (11). Interesting to note that insulin level was not affected by SVS and SR treatment in normoglycemic rats whereas it was improved in hyperglycemic rats. It is presumably that SVS containing in SR potentiated glucose-induced insulin release. This is supported by the evidence that SVS stimulates insulin release from isolated pancreatic islets only in the presence of high glucose concentration (11). Beside the effect on insulin release, SR suppressed an increased glucagon in diabetic rats to the normal level whereas SVS did not modify glucagon level, implying that SVS does not account for the action of SR on glucagon suppression. It can not be explained by the present result why SR decreased plasma glucagon only in diabetic rats without effect on normal rats, and should be further clarified.

The present results show that SVS slightly but significantly raised $P_G$ without effect on both insulin and glucagon level in normal rats whereas it raised insulin without effect on $P_G$ in hyperglycemic rats. An impairment of tissue glucose uptake may be the possible cause. This is supported by the results that muscle response to insulin was depressed in isolated diaphragm muscle of both normal and diabetic rats fed with SVS. Therefore, suppression of insulin-induced glucose uptake seems to be a primary cause of hyperglycemia in normal rats fed with SVS. Though insulin-induced glucose uptake in DM-SVS was depressed, but the level was remained greater than that of normal rats fed with SVS and this may result in an unchanged $P_G$ instead of higher $P_G$ in DM-SVS. It is interesting to note that a reduction of insulin-induced glucose uptake produced by SVS was reversed in diaphragm muscle isolated from both normal and diabetic rats fed with SR. Whether some glycosides containing in SR inhibit the suppressive action of SVS on insulin-induced glucose uptake is not known. More information is needed to clarity the glycemic action of other glycosides containing in SR.

It can be concluded that administration of SVS for 8 weeks raised $P_G$ in normal rats whereas no significant change of $P_G$ in diabetic rats was apparent. In contrast, SR reduced $P_G$ in diabetic rats without effect on $P_G$ in normal rats. There were no changes in serum insulin and glucagon levels in normal rats fed with either SVS or SR. In contrary, SVS and SR improved the serum insulin level to the same extent in diabetic rats. High level of glucagon in diabetic rats was depressed to normal value in diabetic rats fed with SR whereas no change was noted in diabetic rats fed with SVS. Insulin-induced glucose uptake by isolated diaphragm muscle was

suppressed in both normal and diabetic rats treated with SVS but returned to normal level in rats fed with SR. The suppression of insulin-induced glucose uptake seems likely the primary cause of hyperglycemia in normal rats fed with SVS. Although SVS potentiated insulin release in diabetic rats, a reduction of insulin-induced glucose uptake was apparent as well, thereby no anti-hyperglycemic effect was apparent. SR decreased $P_G$ in diabetic rats by the mechanism of an improvement of insulin and suppression of glucagon level. SVS containing in SR may indirectly contribute to anti-hyperglycemic action of SR in diabetic rats via its effect to potentiate insulin release.

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