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Original Research

Effect of *Stevia rebaudiana* Bertoni extract on sexual dysfunction in Streptozotocin-induced diabetic male rats

Matin Ghaheri[1,2], Shahram Miraghaee[1], Atefeh Babaei[1*], Bahareh Mohammadi[1], Danial Kahrizi[1,2], Zahra Minoosh Siavosh Haghighi[4], Gholamreza Bahrami[2,3]

[1] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Department of Agronomy and Plant Breeding, Faculty of Agriculture, Razi University, Kermanshah, Iran
[3] Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Faculty of Veterinary Medicine of Razi universiry. Kermanshah, Iran

Correspondence to: Atefeh_babaie@yahoo.com

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Abstract: *Stevia rebaudiana* Bertoni has been used locally as a non-calorie sweetener in medicine and diabetic diet which claimed to have aphrodisiac properties, although no scientific data of this function have been reported. The aim of this study was to investigate the effect of *S. rebaudiana* extract on sexual dysfunction, testosterone levels and number of Leydig cells in Streptozotocin (STZ)-induced diabetic male rats. A total of 28 diabetic male rats were randomly divided into 4 groups: diabetic group without any extract and 3 extract groups (5, 50 and 100 mg/kg). Seven normal control rats were treated with vehicle mount latency and frequency of (ML, MF), intromission latency and frequency (IL, IF), ejaculation latency and frequency of (EL, EF), the mount latency post ejaculation (MPE), the intromission latency post ejaculation (ILE), the intromission frequency post ejaculation (IFE) were recorded during 30 min on days 0, 14, 28. The serum testosterone levels, blood glucose, sex organs weight, number of leydig cells and histology of testicular tissue were measured. The stevia group (5 mg/kg) had a significant ($p<0.05$) increase in EF and IF. The number of Leydig cells in the diabetic group were significantly ($p<0.05$) reduced compared to the normal group and diabetic groups with extract (5 and 50 mg/kg). The serum testosterone levels and other sexual behaviors did not show any significant differences. The low- dose stevia extract with attention to antioxidant, vasodilator and anti-diabetic properties can be aphrodisiac in STZ- induced diabetic male rats.

Key words: Diabetes; Sexual dysfunction; *Stevia Rebaudiana* Bertoni; Leydig cells; Testosterone levels; Male rats.

Introduction

Diabetes mellitus is a common chronic metabolic disorder that affects the metabolism of carbohydrates, lipids and proteins .Hyperglycemia is caused by altered secretion of insulin and or resistance to insulin action (1). The high level of blood glucose causes damage to the nerve system, blood vessels leading to complications such as erectile dysfunction (2). The erectile dysfunction is defined as the male's ability to obtain and maintain a sufficient penile erection in a satisfactory sexual intercourse (3). The extensive research about humans and laboratory animal models suggested that the diabetes cause weakness, depression, anxiety, reduced volume of ejaculate, hypogonadism, infertility due to the alteration in spermatogenesis, changes in testes structure, alteration in glucose metabolism in Sertoli cells, reduction in the concentrations of testosterone, ejaculatory dysfunction and reduction in libido in males (4-6). Diabetes mellitus may cause erectile dysfunction by pathological changes including neuropathy, endocrine disorders, endothelial dysfunction, changes in the performance and structure of cavernous smooth muscle and increased oxidative stress (5-7). *Stevia rebaudiana* Bertoni is a perennial herb that belongs to the *Asteraceae* family (8) and is used as a natural sweetener plant (without calorie) in medicine and diabetic diets being 300 times sweeter than sugar (9, 10). The sweetener effect arises from the compounds such as Rebaudioside A and Steviol (9). Many healing properties have been proven for Stevia, including: antioxidant, anti-diabetic, vasodilator, antihypertensive, diuretic, immunomodulatory, antimicrobial, memory improvement, cardiotonic, weight loss and anti-depressant effects (8-11). Previous studies have shown that Stevia has increased the level of testosterone and estrogen (12) while, it has been proven that Stevia extract has significantly reduced the weight of testis, seminal vesicles and caudal epididymis (13). It has also been shown that the administration of Rebaudioside A in F_0 and F_1 generations has not affected the reproductive parameters such as mating, fertility, gestation lengths, estrous cycle and sperm motility, morphology (14). However, there are no scientific reports on the effect of stevia on sexual behavior in STZ- induced diabetic male rats. The aim of this study was evaluation the effect of Stevia on sexual function in Streptozotocin-induced male rats.

Materials and Methods

Plant material and preparation of extract

The *S. rebaudiana* plant samples were provided by

Zagros Bioidea Co. Razi University, Kermanshah, Iran. A total of 500 mg of dried leaves was heated in 100 ml of distilled water at 60 °C for 5 min. Then were filtered and macerated in 100 ml of ethanol for 12 hours. The extract was re-filtered and passed through Silica gel column. Finally the extract was concentrated under reduced pressure at 45 °C.

Laboratory animals

In this study 140 adult healthy albino rats of Wistar strain of either sex (38 males and 150 females) were used. Rats were maintained at the animal house of Kermanshah University of Medical science at 22 °C with a light: dark cycle (12h: 12h) and free access to standard laboratory rat diet and water. All animals were treated in accordance with the principle of the laboratory animal care (National Institutes of Health) (15). The experimental protocol was approved by the animal ethical committee in Kermanshah University of Medical science according to guide for care and use of laboratory animals.

Experiment design

Diabetes mellitus was induced by intraperitoneal injection of Streptozotocin (50 mg/kg). After 72 hours, only rats exhibiting a fasting glucose level greater than 250 mg/dl were selected. The level of fasting glucose was measured in days 0, 14 and 28. A number of 35 male rats (28 diabetic and 7 non diabetic) were divided randomly into 5 groups of 7 rats in each group and treated as follows: group 1: non diabetic control group receiving 2 ml distilled water, group 2: diabetic control group receiving 2 ml distilled water and groups 3, 4 and 5: diabetic groups receiving Stevia extract of 5, 50 and 100 mg/kg, respectively. The distilled water and plant extract were orally administered by means of a gastric tubing (2 ml/rat) once a day for 28 days.

Stimulation of females

The estrus was induced in adult female rats by the subcutaneous injection of 20 µg estradiol benzoate (48 hours prior to testing) and progesterone (4 hours prior to testing). Before the test, only female rats exhibiting good sexual receptivity were selected for this study (5-6, 16).

Sexual behavior tests

Before the induction of diabetes, all male rats were exposed to the appropriate receptive female rats for 10 min to stimulate mating behavior. Only rats that mounted within 10 min were selected. The sexual behaviors were recorded by the camera for 30 min in a separate room on days 0 (72 hours after STZ injection), 14 and 28. The sexual behavior parameters including: mount latency (ML), intromission latency (IL) (defined as times from introduction of the female in the cage to the first mount and intromission), mount frequency (MF) (the number of mount and intromissions preceding the first ejaculation), intromission frequency (IF), ejaculation latency (EL) (the time from introduction of the female in the cage to the first ejaculation), the mount latency post ejaculation (MLE), the intromission latency post ejaculation (ILE), intromission frequency post ejaculation (IFE) (the number of intromission after the first ejaculation) and ejaculation frequency (EF) were recorded (Figure 1) (5-6, 16).

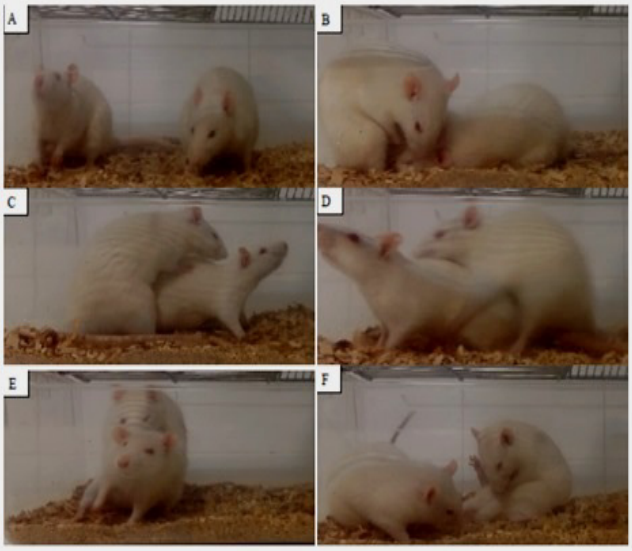

Figure 1. Photographs of rats displaying sexual behaviors: (A) Environment Identify, (B) Annoy, (C) Mount, (D) Ejaculation, (E) Intromission, (F) cleaning himself.

Serum testosterone measurement

At the end of the experiment, animals were sacrificed under anesthesia and blood samples were collected and centrifuged at 4000 rpm, 4 °C for 10 min for serum separation. The serum samples were stored at -35 °C untill hormonal analysis. Total serum concentration of testosterone from male rats was measured by radioimmunoassay (16).

Histology

The right testis and epididym were removed and weighted then fixed in 10% formalin. After histological processing, 5µm- thick paraffin sections were stained with Hematoxin & Eosin. The images of tissues were photographed and analyzed using a camera-mounted microscope (17).

Statistical analysis

Statistical analyses were carried out by SPSS16 software (SPSS/PC-16.SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± SEM. The treated groups were compared to control by ANOVA followed by Turkey's post-hoc test. The $p<0.05$ was considered statistically significant.

Results

Effect on fasting blood glucose level and weight of sexual organs

Table 1 shown that 72 hours after STZ injection, blood glucose levels in all diabetic rats were significantly ($p<0.05$) increased compared to the normal control group. The level of glucose in Stevia groups (5 and 100 mg/kg) was significantly ($p<0.05$) decreased after 4 weeks of Stevia administration. After 28 days of the treatment, none of the diabetic rats in Stevia groups (5, 50 and 100 mg/kg), showed a significant ($p<0.05$) difference in blood glucose levels compared to the normal rats. In this study was not observed Any significant ($p<0.05$) differences on the weight of testes and epidi-

Table 1. Effect of extract of *S.rebaudiana* on weight of testis and epididymis in diabetic rats.

Treated groups	Blood glucose (mg/dl)			Weight of organs (mg/kg BW)	
	0 Day	14 Day	28 Day	Testes	Epididymis
Control	115.666±16.280[b]	133.500±15.456[b]	140.000±17.235[b]	4.270±0.758	1.907±0.248
STZ	398.400±96.606[a]	448.800±42.364[ac]	327.20±31.655[a]	4.683±0.345	1.777±0.096
STZ+S5mg/kg	326.66±45.660[a]	285.666±80.961[b]	269.50±61.179[bc]	3.286±0.502	2.576±0.492
STZ+S50 mg/kg	380.800±39.114[a]	450.800±30.882[ac]	327.20±25.685[ac]	4.622±0.404	1.780±0.166
STZ+S100 mg/kg	326.666±32.218a	329.166±35.035[b]	205.50±31.589[bc]	4.177±0.324	1.611±0.032

Mean ± S.E.M, P<0.05, n=7. Value in each row marked different superscript letter differs significantly.

dym were observed between all groups (Table1).

Effect on Sexual behavior Parameter

Effect on frequency of Mount, Intromission and Ejaculation

As shown in table 2, after 28 days treatment on diabetic rats with Stevia extract (5 mg/kg) the frequency of intromission and ejaculation was significantly (p<0.05) increased compared to the diabetic rats. No significant (p>0.05) differences were observed on MF, MFE and IFE between examined groups.

Effect on Latency of Mount, Intromission and Ejaculation

The time first performed of mount (ML), intromission (IL) and first intromission after first ejaculation (ILE) were not observed significant different at 0, 14 and 28 days, but the Latency of ejaculation (EL) in diabetic rat group after 28 days administration whit does 5 mg/kg decreased significantly compare with diabetic group and does 100mg/kg of extract (P> 0.05) (Table 2).

Effect on number of Leydig cells

The numbers of Leydig cells per slide were counted in 10 fields. The number of Leydig cells in the diabetic and 100 mg/kg of Stevia treated groups was significantly (P< 0.05) decreased compared to the normal group but after 28 days treatment in diabetic rats with Stevia extract (5 and 50 mg/kg) showed no significant differences compared to the normal group (P> 0.05) (Figure 2, 3).

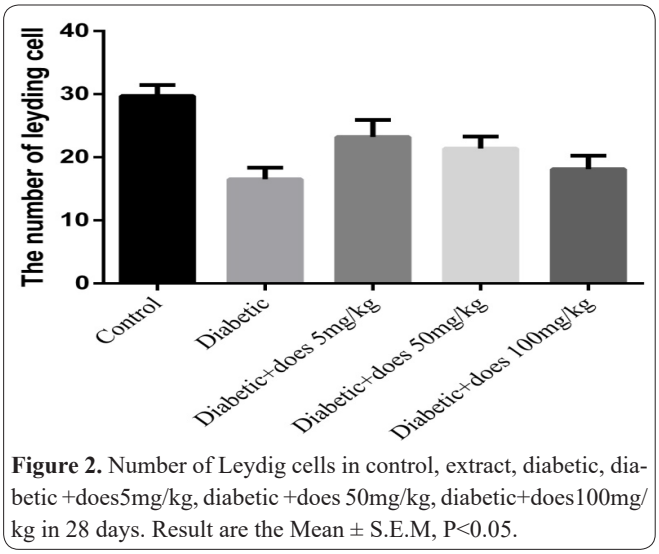

Figure 2. Number of Leydig cells in control, extract, diabetic, diabetic +does5mg/kg, diabetic +does 50mg/kg, diabetic+does100mg/kg in 28 days. Result are the Mean ± S.E.M, P<0.05.

Effect on serum testosterone

The levels of testosterone in the blood serum of control, diabetic and Stevia groups are presented in figure 4. Although after 28 days, the levels of testosterone

Table 2. Effect of extract of *S.rebaudina* on frequency of sexual behavior in diabetic rats.

Parameters	Control	STZ	Treated groups STZ+S5mg/kg	STZ+S50 mg/kg	STZ+S100 mg/kg
MF					
0 day	7.000±2.702	3.833±1.108	7.500±5.319	4.143±1.5800	6.285±0.805
15 day	5.200±0.800	5.333±0.666	5.500±0.619	2.429±0.685	4.714±1.106
28 day	3.400±1.122	5.166±2.151	5.000±1.316	4.857±1.388	6.428±2.080
IF					
0 day	12.200±4.715	11.166±3.320	16.666±1.173	18.857±4.273	16.571±3.524
15 day	16.600±7.782	11.500±2.813	24.500±5.578	14.143±7.152	13.000±5.678
28 day	11.200±6.406[ab]	5.833±1.887[b]	25.667±2.139[a]	15.000±5.066[ab]	12.285±4.252[ab]
EF					
0 day	2.400±0.400	2.333±0.494	1.833±0.307	1.571±0.202	1.143±0.143
15 day	1.800±0.374	1.166±0.166	2.166±0.307	1.429±0.429	1.429±0.202
28 day	2.000±0.548[ab]	1.333±0.211[b]	2.666±0.211[a]	1.714±0.285[ab]	1.143±0.143[b]
MFE					
0 day	3.800±1.241	2.500±1.118	2.667±0.760	1.714±0. 565	1.143±0.143
15 day	2.800±1.114	2.000±0.816	3.500±0.763	1.143±0.143	2.575±0.869
28 day	2.666±1.666	2.666±1.475	3.500±0.846	2.000±0.488	2.714±1.714
IFE					
0 day	4.000±1.732	3.333±1.173	8.666±3.158	6.857±1.641	1.1423±0.143
15 day	8.000±4.289	2.833±1.470	10.1667±4.377	4.857±3.857	5.286±2.437
28 day	8.800±4.841	3.166±1.514	14.333±1.358	10.000±4.023	4.571±3.571

Mean ± S.E.M, P<0.05, n=7. Value in each row marked different superscript letter differs significantly.

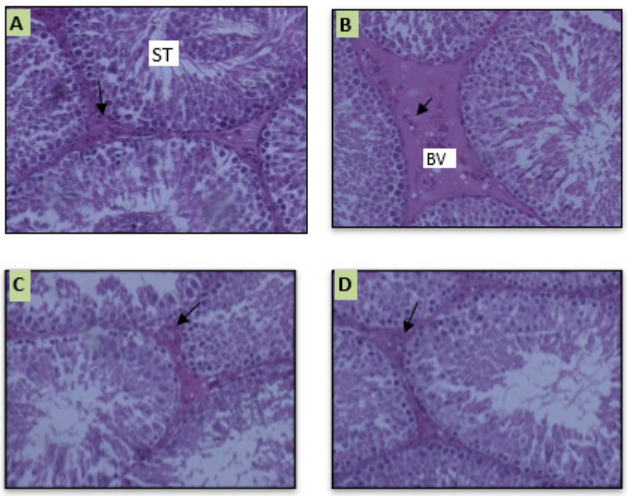

Figure 3. Photomicrographs for section of testis of the control group (A), diabetic group (B), diabetic group+ does 5 mg/kg Stevia (C) and diabetic group+ does 100 mg/kg Stevia (D) presenting the interstitial compartment and presence of Leydig cell (black arrow). Somniferous tubule (ST), Blood vessel (V).(400×).

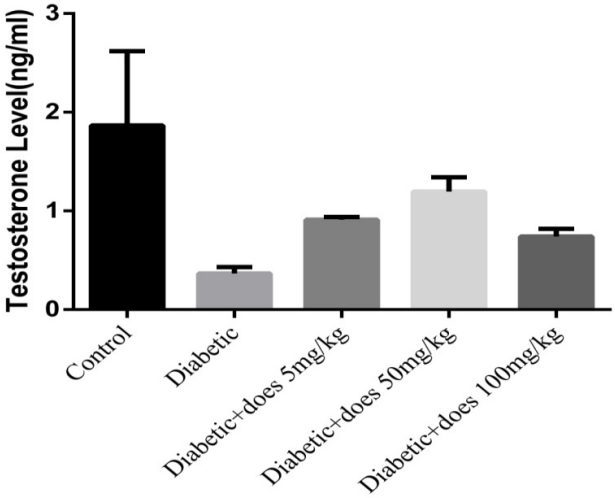

Figure 4. Testosterone level in control, diabetic, diabetic +does 5 mg/kg, diabetic + does 50 mg/kg, diabetic + does100 mg/kg in 28 days. Result are the Mean ± S.E.M, P<0.05.

in the normal and Stevia groups were more than diabetic group, but this increase was not statistically significant (P> 0.05) (Figure 4).

Discussion

Streptozotocin has been used as a tool for induced diabetes in laboratory animals (18). Diabetes is associated with cardiovascular, obesity, neurological and reproduction disorders (4, 19, 20). Reproductive disorders in diabetic patient including: depression, anxiety, ejaculation dysfunction, decrease in levels of testosterone, progressive of endothelium tissue and smooth muscle tissue, damage to regulation of smooth muscle tone, decrease in number of Leydig cells (5, 19, 20). The main purpose of this study was to evaluate of effect of Stevia extract on sexual behavior dysfunction in STZ-induced diabetic rats. This study clearly proved that STZ- induced diabetic male rats caused reduces in blood glucose levels, sexual behavior dysfunction, decrease in testosterone levels, erection dysfunction, intromission dysfunction and decrease in number of Leydig cells ($p<0.05$). Furthermore, after 4 weeks treatment with Stevia extract in diabetic rats caused decrease in levels of blood glucose, improvement sexual behavior dysfunction, increase in testosterone levels and number of Leydig cells ($p<0.05$). The previous studies have shown that atherosclerosis and endothelial dysfunction in diabetes may limit the necessary blood flow of arterial for erection to the penis (21). Several finding have suggested that Stevia cause vasodilation and increase blood flow (22), which maybe increase blood flow to penis vessels and help to improve the erectile performance. On the other hand, the anti-diabetic properties of Stevia in various studies (23) and present study have been proven. Many of sexual dysfunction related to diabetes is due sustained hyperglycemia and its complication (5). The administration of Stevia reduced blood glucose levels in diabetic rats and maybe increased the complication of hyperglycemia. Different studies have shown that oxidative stress is a major role to erectile dysfunction and decreases the number of Leydig cells in

Table 3. Effect of extract of *S.rebaudina* on latency of sexual behavior in diabetic rats.

		Treated groups			
Parameters	**Control**	**STZ**	**STZ+S5mg/kg**	**STZ+S50 mg/kg**	**STZ+S100 mg/kg**
ML					
0 day	793.000±411.124	833.500±325.589	203.000±99.166	929.000±319.166	272.570±134.903
15 day	300.00±132.81	432.830±189.510	255.170±61.180	938.570±313.770	826.86±301.42
28 day	486.00±330.37	934.17±236.39	456.500±113.47	732.71±37.762	747.57±281.05
IL					
0 day	104.400±26.763	446.700±274.408	175.830±58.749	175.00±80.197	641.43±247.651
15 day	436.00±164.730	459.000±272.243	309.830±109.889	641.500±167.652	602.57±281.00
28 day	714.000±315.283	1056.000±244.731	408.330±244.731	761.140±278.666	758.710±276.259
EL					
0 day	956.200±305.534	1050.000±251.582	1158.200±245.830	1127.000±259.501	1785.6±144.3
15 day	1332.000±288.000	1307.770±313.101	1176.300±204.503	1602.400±197.571	1545.70±196.336
28 day	1426.000±229.295^{ab}	156.083±177.878^{ab}	881.670±164.319^{b}	1311.100±184.304^{ab}	177.270±272.857^{a}
ILE					
0 day	1186.800±308.377	1209.000±22.583	1560.300±182.849	1415.400±251.563	1785.6±144.28
15 day	1584.000±152.499	1580.200±152.272	1423.500±179.739	1668.400±131.517	1634.700±164.288
28 day	1447.600±260.426	1680.000±120.000	1333.000±107.492	1584.700±123.479	1785.600±14.428

Mean ± S.E.M, P<0.05, n=7. Value in each row marked different superscript letter differs significantly.

diabetic patients and the use of antioxidants can affective on reduce of these damages (24). Many chemical molecules are known to possess antioxidant potentials responsible for induced the endothelium dysfunction by oxidative stress through the regeneration of antioxidant enzymes (5). The antioxidant properties of Stevia have been proven in the many studies and have been shown that Stevia can reduce degradation in endothelial and Leydig calls with its antioxidant action (5, 25). Our results were confirmed that Stevia prevented the destruction of Leydig cells in treated diabetic rats compare to untreated diabetic rats. Since Stevia is a non-caloric sweetener, anti-diabetic, vasodilator, antioxidant and our study showed that Stevia is herb with reduce of blood glucose and aphrodisiac property and can be effective for treatment of erectile and intromission dysfunction in diabetic patient.

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