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RESEARCH ARTICLE

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The effect of stevia sweetener on diabetic nephropathy in male albino rats

ABSTRACT:

Diabetic nephropathy (DN) is considered a clinical syndrome, which is a diabetic kidney disease characterized by increasing blood pressure, albuminuria, and chronic kidney disease. The current study aimed to determine the effect of sweetener (*Stevia rebaudiana*) on diabetic nephropathy in a diabetic rat model. Four groups (n = 7) of male albino rats (*Rattus norvegicus*): the control group (healthy); the other three groups were diabetic (induced streptozotocin intraperitoneally), one as non-treated and two diabetic rat groups treated with 2 doses of stevia (200 and 400 mg/kg body weight, respectively) daily for 4 weeks. Biomarkers of nephropathy were measured in plasma and kidneys at the end of the experiment. The levels of plasma glucose, urea, uric acid, creatinine, renal malondialdehyde (MDA), and caspase-3 significantly increased in diabetic rats and levels of insulin, renal catalase, superoxide dismutase, and vascular endothelial growth factor A (VEGF-A) were significantly decreased compared to the healthy control group. After treating the diabetic rats with natural sweetener (stevia), the kidney and plasma biochemical parameters were improved significantly. These results indicated the major role of stevia in controlling diabetic nephropathy.

KEY WORDS:

Diabetic nephropathy, natural sweetener, *Rattus norvegicus,* Stevia, Streptozotocin.

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INTRODUCTION:

Diabetes mellitus is considered a metabolic illness, caused by insufficient insulin secretion, resistance to insulin action, or both (Ahmad and Ahmad, 2018). Diabetic nephropathy is a complication of diabetes that caused a loss in kidney functions and damage to blood vessels ending in death (Moresco *et al*., 2013). Reactive oxygen species (ROS) are overproduced due to hyperglycaemia and by using antioxidants has an important role in controlling DN (Wagener *et al.,* 2009). There are numerous adverse impacts associated with the medications used to treat diabetes, so that, using herbal therapy is more preferred because of its lower cost, antioxidant effects, and has fewer or no adverse impacts (Abdel-Aal *et al*., 2021). *Stevia rebaudiana* is a traditional plant that is well-known for its sweet flavour and beneficial impact on the regulation of blood sugar (Assaei *et al*., 2016).

Stevia is known as a sweet herb honey leaf that is from the Asteraceae family (Anbazhagan *et al*., 2010). Stevia leaves contained glycosides, such as stevioside, rebaudiosides, steviolbioside, and dulcoside A, but the main sweet components are rebaudioside A and stevioside (Brahmachari *et al*., 2011; Lemus-Mondaca *et al*., 2012). Stevia is considered a non-caloric sweetener and the stevioside is the major sweet constituent in *S. rebaudiana* leaves which is more sweetener than sucrose about 300 times (Ferrazzano *et al*., 2016). It has antioxidant, antihypertensive, antihyperglycemic, anti-hypertensive anti-carcinogenic, antidiarrheal, immunomodulatory, and gastroprotective properties (Ahmad and Ahmad, 2018). The present study aims to evaluate the effects of two doses (200 and 400 mg/kg bw) of stevia (*Stevia rebaudiana Bertoni*) leaves on hyperglycaemia and different physiological parameters in diabetic rats.

MATERIAL AND METHODS:

Sweetener:

The stevia (*Stevia rebaudiana*) leaf powder was obtained from the brand Teyab Shana and dissolved in distilled water.

Animals:

Twenty-eight male albino rats (140 ± 10g) from The Animal Farm, Faculty of Veterinary Medicine, Zagazig University. Animals acclimatized for 10 days under the laboratory conditions (12 hours light/dark cycle and 24 ± 2°C) before used in the current experiments.

Induction of diabetes mellitus:

Twenty-one rats were injected with an intra-peritoneal dose of (45 mg/kg bw, dissolved in 0.01 M citrate buffer at pH 4.5) of streptozotocin (STZ, Sigma Co., USA) after 12 h fasting. The concentrations of blood glucose were examined in the third day and rats having glucose concentrations exceeding 200 mg/dl were diabetic and used for our study.

The design of the experiment:

Four groups of rats (each of seven rats) were prepared. The control group (Group I) was the healthy rats, the other three groups were the diabetic rats and were designed as: a non-treated diabetic group (Group II), and two treated diabetic groups (Group III and Group IV) were given stevia leaves powder (200 and 400 mg/kg body weight suspended in water, respectively) daily for 4 weeks. The study was approved by Benha University for Animal Care and Use Committee (ZD/FSc/BU-IACUC/2022-12), Faculty of Science, Zoology Department. At the end of the experiment, rats were fasted overnight and anesthetized by isoflurane inhalation. Blood was taken from the postcaval vein and transported to tubes containing EDTA (ethylenediamine tetra-acetic acid) from El-Gomhorya Co., Egypt. The tubes were centrifuged at 1500 *Xg* for fifteen minutes, then plasma was separated and stored at - 20°C. The kidneys were removed from rats and washed in ice-cold phosphate buffer saline (PBS) at pH 7.3 then the samples were homogenized in the PBS and centrifuged at 4000 *X*g for five min and the supernatants were stored for assay.

The biochemical analysis:

The blood glucose level was measured by using a Spinreact (Spain) kit, and the level of insulin was measured by using a sandwich ELISA rat kit purchased from BioVendor Research and Diagnostic Products (Japan), urea nitrogen and creatinine were estimated by using a kit from Diamond Diagnostics (Egypt), uric acid was estimated using a kit purchased from Spinreact (Spain), renal malondialdehyde content and catalase activity were estimated by kits from Biodiagnostic, Giza, Egypt, renal superoxide dismutase activity was estimated by using a kit from BioVision (USA). Tumor necrosis factor-alpha, interleukin-6, vascular endothelial growth factor A and caspase-3 were estimated by ELISA rat kits purchased from the Cloud Clone Corporation (USA).

Statistical analysis:

Results were analysed by using the one-way- analysis of variance (ANOVA) followed by Duncan´s test by the Statistical Package for Social Science SPSS (version 20) program (IBM Software, Inc. Chicago, IL, USA) as the mean of 7 individual values ± the standard deviation “SD”.

RESULTS:

The impact of stevia on blood glucose and insulin in diabetic rats:

In the diabetic rats, the blood glucose level was increased significantly ($P < 0.05$), and plasma insulin level was decreased significantly compared to the healthy normal rats. On the other hand, blood glucose level was reduced, and plasma insulin level was elevated in treated diabetic rats in comparison with non-treated ones. The higher dose of stevia (400 mg/kg bw) decreased blood glucose levels and increased insulin levels more than the lower dose (200 mg/kg bw) (Table 1).

Table1. Blood glucose and insulin in normal (Gp I), diabetic (Gp II), and diabetic rats that treated with two doses (200 and 400 mg/ kg bw) of stevia (*Stevia rebaudiana*) (Gps III and IV, respectively).

	Group I	Group II	Group III	Group IV
Glucose (mg/dL)	105.4 ± 0.6^{d}	272.1 ± 0.8^{a}	145.3 ± 0.4^{b}	120.1 ± 0.6^{c}
Insulin (mg/dL)	2.6 ± 0.6^{a}	0.7 ± 0.4^{d}	1.7 ± 0.8^{c}	2.1 ± 0.8^{b}

Each value is mean ± SD for seven rats in each group. The values in the same raw with different small letters (a, b, c, and d) are significantly different at $p < 0.05$.

The impact of stevia on renal function parameters:

The levels of blood urea nitrogen, creatinine, and uric acid were significantly increased, ($P < 0.05$) in diabetic rats (group II) comparing with those in normal rats (group I). Whereas these levels were decreased significantly in treated diabetic rats compared to those of the non-treated diabetic rats. The high dose of stevia was more effective than the lower dose; and there was no significant difference between group IV of the high dose and the control healthy group I (Table 2).

Table 2. Kidney functions in normal (Gp I), diabetic (Gp II) and diabetic rats treated with two doses (200 and 400 mg/ kg bw) of stevia (*Stevia rebaudiana*) (Gps III and IV, respectively).

	Group I	Group II	Group III	Group IV
Urea nitrogen (mg/dL)	30.2 ± 0.9^{c}	53.2 ± 0.2^{a}	49.3 ± 0.5^{b}	37.4 ± 0.6^{c}
Uric acid (mg/dL)	1.2 ± 0.2^{b}	2.9 ± 0.1^{a}	1.8 ± 0.4^{b}	1.4 ± 0.1^{b}
Creatinine (mg/dL)	0.3 ± 0.09^{c}	1.9 ± 0.05^{a}	0.9 ± 0.09^{b}	0.45 ± 0.06^{c}

Each value is mean ± SD for seven rats in each group. The values in the same raw with different small letters (a, b, c, and d) are significantly different at $p < 0.05$.

The impact of stevia on oxidative stress:

The level of renal MDA showed a significantly increased ($P < 0.05$), and the activities of renal CAT and SOD showed significantly decreased in the diabetic group II in comparison with those in normal control rats. On the other hand, renal MDA levels were reduced, and renal CAT and SOD activities were elevated in groups III & IV after treatment with stevia in comparison with those in diabetic rats' group II (Table 3).

Table 3. Oxidative stress parameters in normal (Gp I), diabetic (Gp II), and diabetic rats treated with two doses (200 and 400 mg/ kg bw) of stevia (*Stevia rebaudiana*) (Gps III and IV, respectively).

	Gp I	Gp II	Gp III	Gp IV
SOD (U/mg protein)	3.60 ± 0.32^{a}	0.51 ± 0.01^{c}	2.40 ± 0.39^{b}	3.21 ± 0.01^{a}
CAT (nmol/mg protein)	2.60 ± 0.04^{a}	0.87 ± 0.04^{c}	1.72 ± 0.18^{b}	2.42 ± 1.66^{a}
MDA (nmol/mg protein)	0.91 ± 0.02^{c}	3.61 ± 0.11^{a}	2.11 ± 0.11^{b}	1.22 ± 0.17^{c}

Each value is mean ± SD for seven rats in each group. The values in the same raw with different small letters (a, b, c, and d) are significantly different at p < 0.05.

Effect of stevia on inflammatory cytokines in diabetic rats:

In the diabetic rats, the levels of TNF-a and IL-6 were increased significantly in comparison with those in normal rats and they decreased significantly in treated diabetic rats (group III & IV) compared to those in non-treated ones (group II). The dose of stevia (400 mg/kg bw) was more effective than the lower dose; there was no significant difference between the group of high dose and the control normal rats (Table 4).

Table 4. Proinflammatory cytokines parameters in normal (Gp I), diabetic (Gp II), and diabetic rats treated with two doses (200 and 400 mg/ kg bw) of stevia (*Stevia rebaudiana*) (Gps III and IV, respectively).

	Group I	Group II	Group III	Group IV
TNF-α (ng/ml homogenate)	27.6 ± 3.7^{c}	39.1 ± 5.8^{a}	33.0 ± 2.5^{b}	29.6 ± 0.8^{c}
IL-6 (Pg/mg protein)	17.3 ± 0.2^{c}	33.4 ± 0.7^{a}	26.0 ± 0.9^{b}	19.6 ± 0.7^{c}

Each value is mean ± SD for six rats in each group. The values in the same raw with different small letters (a, b, c, and d) are significantly different at $p < 0.05$.

Effect of stevia extract on caspase-3 and VEGF-A in diabetic rats:

The data presented in table 5 indicated that in diabetic rats, the activity of renal caspase-3 was elevated significantly, and renal VEGF-A levels reduced significantly compared with those in normal rats. On the other hand, renal caspase-3 activity reduced significantly, and renal VEGF-A levels elevated significantly in treated diabetic rats compared to those in diabetic rats (group II). As mentioned above, the high dose of stevia (400 mg/kg bw) was more effective than the lower dose (200 mg/kg bw) and there was no significant difference between those treated with the high dose and the control rats.

Table 5. Renal caspase-3 and vascular endothelial growth factor A levels in normal (Gp I), diabetic (Gp II), and diabetic rats treated with two doses (200 and 400 mg/ kg bw) of stevia (*Stevia rebaudiana*) (Gps III and IV, respectively).

	Group I	Group II	Group III	Group IV
CASP-3 (Pg/mg protein)	17.6 ± 3.7^{d}	47.1 ± 5.8^{a}	32.0 ± 2.5^{b}	25.6 ± 0.8^{c}
VEGF-A (Pg/mg protein)	75.3 ± 0.2^{a}	57.4 ± 0.7^{d}	63.0 ± 0.9^{c}	69.6 ± 0.7^{b}

Each value is mean ± SD for six rats in each group. The values in the same raw with different small letters (a, b, c, and d) are significantly different at $p < 0.05$.

DISCUSSION:

The major cause of diabetic nephropathy is hyperglycaemia (Mima, 2013). *Stevia rebaudiana* is a well-known medicinal herb that is used in different medications around the world. The present study was carried out to explore additional therapeutic impacts of stevia in a diabetic rat model by using low and high doses. The results of our study indicated the hypoglycaemic effect of stevia on diabetic rats by using low and high doses. Present study indicated that using (400 mg/kg bw) of stevia had a perfect impact in controlling diabetes and fasting blood glucose levels and insulin secretion in

diabetic rats for 4 weeks. These was probably due to phytochemicals (Steviol, Steviosides, rebaudiosides, etc.) especially stevioside which may help in reducing blood sugar levels by stimulating the insulin receptor to insulin or sensitize the β-cells of islets of Langerhans to release insulin which leads to normal blood glucose level (Ahmad and Ahmad, 2018; Jan *et al*., 2021). In our present study, stevia decreased blood urea nitrogen, uric acid, and creatinine levels in diabetic rats which are renal function markers as diabetic nephropathy is characterized by elevated levels of serum creatinine, and blood urea nitrogen (Zou *et al*., 2014). Thus, present results indicated that stevia ameliorated renal dysfunctions biomarkers in diabetic nephropathy. Oxidative stress, which caused due to an unbalance between reactive oxygen species (ROS) production and the antioxidant enzymes and excessive ROS production causes antioxidant depletion (Tousson *et al*., 2019). The results of present study indicated that the level of the indicator of ROS (renal MDA) increased, but the activities of antioxidant enzymes (renal SOD and CAT) decreased in diabetic rats compared to normal rats. These results may be due to hyperglycaemia which in diabetic nephropathy (DN) increased the production of ROS in the mesangial and tubular epithelial cells (Arora and Singh, 2013). The present study revealed a significant decrease in the level of renal MDA and a significant increase in the activities of renal SOD and CAT in treated diabetic rats compared to diabetic ones. The antioxidative properties present in the leaves of *Stevia rebaudiana* may be due to high phenols and flavonoid contents (Assaei *et al*., 2016).

Interleukin 6 (IL-6) and tumour necrosis factor α- (TNF-α) are considered an inflammatory biomarker used for the early diagnosis of diabetic nephropathy (Moresco *et al*., 2013). The current study revealed a significant increase in renal IL-6 and TNF- α levels in diabetic rats in comparison with normal rats which are considered inflammatory biomarker used for the early diagnosis of diabetic nephropathy and causes renal injury and disruption of the glomerular permeability barrier (Moresco *et al*., 2013). These results might be due to the increase of ROS that increases the inflammatory cytokines (Wagener *et al*., 2009). Successfully, the present study indicated a significant decrease in renal IL-6 and TNF-a levels in treated diabetic rats compared to diabetic and there was no significant difference between the control group and diabetic rats treated with 400 mg/kg of stevia. These results may be due to the presence of stevioside and steviol, the two compounds that reduce inflammation by stimulation and upregulation of the *IκBα* gene (NF-*κ*B localization inhibitor) (Jan *et al*., 2021).

In the current study, the activity of renal caspase-3 increased, and the level of VEGF-A decreased significantly in diabetic rats which may be due to elevated renal ROS levels that enhanced renal apoptosis in diabetic rats and the development of diabetic nephropathy (Wagener *et al*., 2009). In treated diabetic rats in present work, the activity of caspase- 3 decreased and the level of VEGF-A increased significantly which might be due to decreasing renal ROS and its antioxidant, and anti-inflammatory effects (El Nashar *et al*., 2022).

In conclusion, the present results revealed the potential effects of stevia as an adjuvant in diabetic nephropathy through potentiating improvement in renal functions, antioxidant, anti-inflammatory, and anti-apoptotic effects.

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تأثير الأستيفيا على اعتلال الكلية السكرى في ذكور الجرذان المَهقاء

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اعتلال الكلية السكرى يعنبر من مضاعفات مرض السكر ويتميز بارتفاع ضغط الدم والبول المحتوى على الالبيومين ومرض الكلى المزمن. تم توزيع اربع مجموعات (ن=7) من ذكور الجِرذان المهقاء (*Rattus norvegicus*) على النحو التالي: مجموعة التحكم، ومجموعة الجرذان المصابة بداء السكرى، ومجموعتان من الجرذان المصابة بداء السكري والتي عولجت بالاستيفيا 200 و 400 ملجم / كجم من وزن الجسم (يوميا لمدة 4 اسابيع، على التوالي). وقد أظهرت الجِرذان المصابة بمرض السكرى زيادات ملحوظة إحصائيًا مقارنة بالمجموعة الضابطة في مستويات سكر الدم واليوريا وحمض البوليك والكرياتينين والمالوندای ألدهيد (MDA) وعامل نخر الورم االفا -(TNF α)والانترلوكين-6 (IL -6) والكاسبيز-3 (Caspase-3) وانخفاض معنويًا فى الكتاليز (CAT) والسوبر اوكسيد ديسميوتيز (SOD) وعامل نمو بطانة الاوعية الدموية (VEGF). وقد أدت المعاملة بسكر الاستيفيا إلى تعديل جميع التغيرات في الجِرذان المصابة بمرض السكرى والخلاصة أن الاستيفيا لها دور محتمل/فعال في تخفيف الاضطرابات البيوكيميائية الناتجة عن اعتلال الكلية السكرى.