Stevia (*Stevia rebaudiana*) leaves: chemical composition, bioactive compounds, antioxidant activities, antihyperglycemic and antiatherogenic effects

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Abstract:

The present study aims to evaluate the chemical composition, bioactive compounds, antioxidant activities, antihyperglycemic and antiatherogenic effects of stevia (*Stevia rebaudiana*) leaves. Total protein, crude fat, ash, crude fiber and carbohydrates in stevia leaves powder (SLP) were recorded 9.63 ± 1.86, 3.47 ± 0.73, 3.08 ± 0.55, 17.12 ± 3.10 and 66.50 ± 3.75 (g/100g DW), respectively. Also, the reducing sugar was registered 4.98 ± 1.12 g/100g DW. Contents of glycosides/sweeteners in SLP (%) were showed stevioside, rebaudioside A and rebaudioside C accounts for 8.87± 1.28, 2.65± 0.24 and 0.43± 0.11 of all glycosides in stevia. Stevia leaves aqueous extract showed high antioxidant activity (AA= 82.05%) when compared with the standard antioxidants (Butylhydroxytoluene, BHT; α-Toc, alphatocopherol). Comparing with the antioxidants standard used, the values of SLAE extracts absorbance’s through 120 min (β-carotene bleaching assay based on measured the ability of an antioxidant to inhibit lipid peroxidation) are coming well i.e. closing the line of 100 mg/L of α-tocopherol and 50 mg/L of BHT standards and up to the line of 50 mg/L of BHT standard.

Feeding diabetic animals with 1, 2, 3 and 4% w/w of SLP caused a significant *p*≤0.05 decrease in serum glucose concentration by the ratio of 70.33, 58.41, 46.79 and 38.46% (as a percent change of the control positive), respectively. Also, the same behavior was recorded for serum level of thiobarbituric reactive substances (TBARS), the biomarker of oxidative stress and the opposite direction was recorded for levels of serum reduced glutathione (GSH), the biomarker of antioxidant defense system in the body. Furthermore, inhibitive action of SLAE against CuSO₄-induced LDL oxidation, which plays a substantially role in atherosclerosis process, was observed. In conclusion, these findings provide a basis for the use of the SLP for the prevention and early treatment of T2DM. Also, the SLAE could be used successfully as a promising tool in the prevention of atherosclerosis through inhibiting LDL oxidation process.

**Key words:** Stevia leaves powder, stevia leaves aqueous extract, streptozotocin, oxidative stress, GSH, LDL oxidation, atherosclerosis.

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Introduction:

Stevia (*Stevia rebaudiana* Bertoni) is a branched bushy shrub of the *Asteraceae* family. It is a nutrient rich herb containing substantial amount of nutrients such as protein, fiber, amino acids, sugars, lipids, essential oils, ascorbic acid, β-carotene, riboflavin, thiamine, minerals (chromium, cobalt, magnesium, iron, potassium, phosphorus) and bioactive compounds including austroinulin, sterebins A-H, nilacin, rebaudi oxides, gibberellic acid, indole-3-acetonitrile, apigenin, quercetin, isoquercitrin, luteolin, Miocene, kaempferol, stigmasterol, xanthophyllus, umbeliferone, chlorogenic acid, caffeic acid, dicaffeoylquinic acid, (Jayaraman *et al*., 2008; Esmat and Ferial, 2009; Hu *et al*., 2010). Therefore, stevia is commercially well known to exert beneficial effects on human health and has become an interesting area of research these days.

Leaves of *S. rebaudiana* has many medical applications such as antimicrobial antiviral, antifungal, anti-hypertensive, anti-hyperglycaemic, anti-tumour, anti-inflammatory, anti-diarrhoeal, anti-human rotavirus activities, anti-HIV, hepatoprotective and immunomodulatory effects (Takahashi *et al*., 1998; Takahashi *et al*., 2001; Hsieh *et al*., 2003; Benfordet *et al*., 2006; Satishkumaret *et al*., 2008; Silva *et al*., 2008; Kedik *et al*., 2009; Mohan and Robert, 2009).

![Fig. (1): Stevia, Stevia rebaudiana Bertoni](image-url)
Stevia products find widespread use in the food industry, such as soft drinks or fruit, desserts, sauces, delicacies, sweet corn, bread, biscuits and a table sweetener juices (Moussa et al., 2003; Massoud et al., 2005a; Goyal et al., 2010). Stevia diterpenes replace sucrose in cereals (muesli) (Wallin, 2007), pickles (Koyama et al., 2003), yoghurt (Massoud et al., 2005; Amzad-Hossain et al., 2010), candy (Goyal et al., 2010), soybeans (Amzad-Hossain et al., 2010) and soy sauce and seafood (Goyal et al., 2010). In Egypt, the gap between sugar production and consumption becomes obviously large, in year 2010, it was estimated to be 0.843 million tons (Richard and Won, 2011). Nowadays, attention is concentrated upon using stevia in food industries, in order to close the gap between the production and consumption. Also, the stevia plant was recently introduced to Egyptian agriculture in order to produce a natural sweetener than can cover some of the lack of sugar production in Egypt (Alaam, 2007).

Expert committees of FAO/WHO Organizations on food additives (JECFA, 2009) reviewed the safety of steviol glycosides (stevia sweeteners) and established an acceptable daily intake (ADI) of 4 mg/kg body weight (b.w.)/day (expressed as steviol). There were no objection letters from the FDA regarding a specific steviol glycoside (rebauudioside A, purity higher than 97%) after two independent self-conducted generally recognized as safe (GRAS) determinations (FDA, 2008). In April 2010, after considering all data related to stability, degradation products, metabolism and toxicology, the European food safety authority (EFSA, 2010) concluded that steviol glycosides are not carcinogenic, genotoxic or associated with any reproductive or developmental toxicity.

All of the previous studies confirmed the use of stevia leaves for the treatment of common diseases. Also considering the economic resource constraints and cheapness of this plant parts, the present study was aimed to (1) evaluate the chemical composition, bioactive compounds and antioxidant activities of stevia leaves, (2) determine the effects of stevia leaves powder on serum glucose levels of diabetic rats, and (3) investigate the antiatherogenic effects of stevia leaves aqueous extract in vitro.

Materials and Methods:

Materials:

Stevia sweeteners were obtained from AWA for food additives Co. Alexandria, Egypt. Streptozotocin, used for induction of DM among rats, Folin-Ciocalteu reagent, o-phosphoric acid, serine borate buffer (SBB), N-1-(pyrenyl) maleimide (NPM), dithiothreitol (DTT) and reduced glutathione (GSH) were obtained from Sigma Chemical Co., St. Louis, Mo. Casein, as
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main source of protein was purchased from Morgan Company for Chemicals, Cairo, Egypt. Vitamins and salts mixtures, organic solvents and other chemicals in analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

Animals used in this study, adult male albino rats (150-160 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Equipment's:

In the present study a SP Thermo Separation Products Liquid Chromatography (Thermo Separation products, San Jose, CA) was used with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used were a Spherosorb ODC-2 (5 µm, 150 x 4.6 mm I.d.) for reduced glutathione (GSH) and Eclipse plus C<sub>18</sub> column (3.5 µm 4.6x100 mm) (Alltech, Deerfield, IL). Also, absorbance and fluorescence for different assays were measured using Labo-med. Inc. spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively.

Methods:

Proximate chemical composition:

Stevia leaves powder (SLP) samples were analyzed for moisture, protein (TN × 6.25, micro-Kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt semiautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and reducing sugar contents were determined using the methods described in the AOAC (2005). Carbohydrates calculated by differences:

Carbohydrates (%) = 100 - (moisture (%) + protein (%) + fat (%) + Ash (%) + fiber (%)

Qualitative identification of stevia sweeteners:

The stevia sweeteners were separate identified by HPLC as described in Sharma et al. (2005). The analysis was performed by linear gradient over 20 min (84 to 50% CH<sub>3</sub>CN in H<sub>2</sub>O/ pH =5, H<sub>3</sub>PO<sub>4</sub>); flow rate 2.0 ml/min, detection at 220 nm. The amount of stevia sweeteners applied was 70 μl. Stevia sweeteners were identified by its characteristic retention time and UV spectra, and the identification was confirmed by the addition of a standard to the injected samples.
Antioxidant activity (AA) :

Antioxidant activity (AA) of SLP aqueous extract (SLPAE) and standards (α-tocopherol and BHT) was determined according to the method described by Marco (1968). For a typical assay, 1mL of β-carotene solution, 0.2 mg/ml in chloroform, was added to round-bottom flasks (50 ml) containing 0.02 ml of linoleic acid and 0.2 ml of Tween 20. Each mixture was then dosed with 0.2 ml of 80% MeOH (as control) or corresponding SLPAE or standard. After evaporation to dryness under vacuum at room temperature (25°C), oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The SLPAE was then subjected to thermal auto-oxidation at 50°C for 2 hr.

The absorbance of the solution at 470 nm was monitored on a spectrophotometer. All samples were assayed in triplicate. Various concentrations of BHT and α-tocopherol in 80% methanol were used as the control. AA of SLPAE was calculated in four different ways as follow: 1st way) absorbance was plotted against time, as a knit curve, and the absolute value of slope was expressed as antioxidant value (AOX) (Al-Saikhan et al., 1995), 2nd way) antioxidant activity (AA) was all calculated as percent inhibition relative to control using the equation (Al-Saikhan et al., 1995) AA=(R<sub>control</sub>-R<sub>sample</sub>)/R<sub>control</sub> x 100 where: R<sub>control</sub> and R<sub>sample</sub> were the bleaching rates of β-carotene in reactant mixture without antioxidant and with plant part extract, respectively, 3rd way) this method of expression based on the oxidation rate ratio (ORR) was calculated according to the method of Marinova et al., (1994) using the equation [ORR = R<sub>sample</sub>/R<sub>control</sub>] where: R<sub>control</sub> and R<sub>sample</sub> are the same in the previous method, and 4th way) the antioxidant activity coefficient (AAC) was calculated as described by Mallet et al. (1994) [AAC = (Abs<sub>S</sub>120- Abs<sub>C</sub>120)/Abs<sub>C</sub>0- Abs<sub>C</sub>120] x 100 where: Abs<sub>S</sub>120 was the absorbance of the antioxidant mixture at time 120 min, Abs<sub>C</sub>120 was the absorbance of the control at time 120 min, Abs<sub>C</sub>0 was the absorbance of the control at zero time.

β-carotene bleaching (BCB) assay :

For β-carotene bleaching (BCB) assay, antioxidant activity (AA) against time (every 10 min thereafter for 120 min) for the SLPAE was measured/constructed according to Marco (1968). The AA was all calculated as percent of inhibition (bleaching rates of β-carotene in reactant mixture of SLP extracts) relative to control (bleaching rates of β-carotene in reactant mixture of without SLP extract) such as described by Al-Saikhan et al. (1995).
Inhibition of low density lipoprotein (LDL) oxidation:

Inhibition of LDL oxidation was determined according to the method of Princen et al. (1992). Adult male white albino rat, Sprague Dawley strain, serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl DMSO containing various concentrations of the SLPAE. A 20 µl of CuSO4 solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculation the net area under the curve.

Biological Experiments:

Experimental animals:

Normal male albino rats (165± 4.73 g per each) were obtained from Agriculture Research Center, Ministry of Agriculture, Cairo, Egypt.

Basal Diet:

The basic diet prepared according to the following formula as mentioned by AIN (1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by Campbell (1963) while the salt mixture used was formulated according to Hegsted (1941).

Experimental design:

All biological experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=36 rats), were housed individually in wire cages in a room maintained at 25 ± 2 °C and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (36 rats) was injected subcutaneous by streptozotocin to induce diabetic rats then classified into sex sub groups as follow: Group (2): Fed on standard diet only as a positive control (rats with diabetes). Groups (3-6): Fed on standard diet containing 1, 2, 3 and 4% (Stevia leaves powder, SLP), respectively.

Induction of diabetes:

Such as reported by Ji et al. (2011) a diabetic state was induced in normal healthy rats by a single intraperitoneal injection of streptozotocin
(STZ, dissolved in 0.05 M citrate buffer, pH 4.5, immediately before use) at a low dose, 40 mg/kg body weight. One week after injection, fasting blood glucose levels were determined from tail blood using a specific kit (AlGomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt). The rats with fasting blood glucose levels above 130 mg/dl were considered to be diabetics and included in the experiments.

**Blood sampling:**

At the end of experiment period, 28 days, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 1000 g to separate the serum according to Drury and Wallington (1980). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis.

**Hematological analysis:**

**Serum glucose:**

Enzymatic determination of serum glucose was carried out spectrophotometerically according to Yound (1975).

**Reduced glutathione (GSH):**

GSH was determined in serum by using HPLC system according to the method of McFarris and Reed (1987). In brief, 100 µl of aliquot were placed in 2 ml of 10% perchloric acid containing1mM bathophenanthroline disulfonic acid and homogenized (Vortex, Labo-med. Inc., UK). The homogenate was cold centrifuged at 2200 g for 5 min and the internal standard (γ-glutamyl glutamate) was added to the supernatant. A 250 µl aliquot of acidic extract was mixed with 100 µl of 100 mMiodoacetic acid in 0.2 mM cresol purple solution. The acid solution was brought to pH 8.9 by the addition of 0.4 ml of KOH (2 M)/KHCO₃ (2.4 M) and allowed to incubate in the dark at room temperature for 1 h to obtain S-carboxymethyl derivatives. The N-nitrophenol derivatization of the samples were taken overnight at 4°C in the presence of 0.2 ml of 1% 1-fluoro-2, 4-dinitrobenzene and injected onto the HPLC system.

**Thiobarbituric acid reactive substances (TBARS):**

TBARS were measured as described by Buege and Aust (1978). In brief, 500µl of plasma samples were added to 1.0 ml of thiobarbituric acid reagent (TBA), consisting of 15% TCA, 0.375% TBA and 0.01% butylhydroxytoluene (BHT) in 0.25N HCl. A 25 µl of 0.1 M FeSO₄.7H₂O was added and the mixture was heated for 20 min in boiling water. The
samples were centrifuged at 1000 g for 10 min and the absorbance (Abs) was read at 535 nm against a reagent blank. Abs of the samples was compared to a standard curve of known concentrations of malonaldehyde (MDA).

Statistical Analysis:

All parameters determination were done in triplicate and recorded as means ±SD. Statistical analysis was performed with the Student t-test and MINITAB-12 computer program (Minitab Inc., State College, PA).

Results and Discussion:

Chemical composition :

Chemical composition of dried stevia leaves was indicated in Table 1. From such data it could be noticed that total protein, crude fat, ash, crude fiber and carbohydrates were recorded 9.63 ± 1.86, 3.47 ± 0.73, 3.08 ± 0.55, 17.12 ± 3.10 and 66.50± 3.75 (g/100g DW), respectively. Also, the reducing sugar was registered 4.98± 1.12 g/100g DW. Such data are in accordance with that obtained by Abou-Arab et al. (2010) and Serio (2010). Fat content in dry matter of stevia leaves amounts to 1.9 - 4.34 g/100 DW (Abou-Arab et al., 2010; Siddique et al., 2014).

In similar studies, Boonkaewwan et al. (2006) and Abou-Arab et al. (2010) found that carbohydrate contents in dry leaves of stevia ranged from 35.2 to 61.9%. From nutritional point of view, carbohydrates positive action is connected with prebiotic properties promoting proliferation of beneficial intestinal microflora. Stevia leaves contain fructo-oligosaccharides at 4.6% and polysaccharides, which regulate lipid metabolism and control blood sugar level (Braz De Oliveira et al., 2011). It is reviewed by Marcinek and Krejpcio (2015) the chemical composition of stevia leaves changes depending on the degree of their processing. For example, Gasmalla et al, (2014) reported that the chemical composition of stevia plant is also affected by the method of leaf drying.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Value (g/100g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>9.63 ± 1.86</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.47 ± 0.73</td>
</tr>
<tr>
<td>Ash</td>
<td>3.08 ± 0.55</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>17.12 ± 3.10</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>66.50± 3.75</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>4.98± 1.12</td>
</tr>
</tbody>
</table>

Each value represents the mean± SD.
Identification of stevia sweeteners:

Contents of glycosides/sweeteners in stevia leaves (%, DW) were separated and identified by HPLC such as showed in Table 2. The result showed that stevioside, rebaudioside A and rebaudioside C accounts for 8.87±1.28, 2.65±0.24 and 0.43± 0.11 of all glycosides in stevia. Many comparative studies of organoleptic analyses showed that pure stevioside is 300 times sweeter than sucrose at a concentration of 0.4%, 150 times sweeter than sucrose when matching a 4% sucrose solution and 100 times when matching a 10% sucrose solution (Hojden, 2000; Nabors O’Brien, 2012). Rebaudioside A is the most important rebaudioside which recorded 250–450 times sweeter than sucrose (Marcinek and Krejpcio, 2015). Such data are partially accordance with that mentioned by Marcinek and Krejpcio (2015), stevioside accounts for 4 up to 13% all glycosides in stevia. It has a permanent bitter or stringent aftertaste.

Molecules of stevioside are highly stable in aqueous solutions within a broad range of pH (1–10) and temperatures up to 198°C (Marcinek and Krejpcio, 2015). Also, Kroyer (2010) showed that steviosides are stable at various processing and storage conditions and in interactions with water-soluble vitamins, organic acids, sweeteners and coffee. Furthermore, during thermal processing they do not participate in the Millard reactions and do not ferment. So, we could concluded that the most important components of stevia include Stevioside (steviol glycoside), which thanks to their high sweetening potential facilitate production of foodstuffs with a reduced energy value.

Table (2): Contents of glycosides/sweetners in stevia leaves (%, DW)

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Content (%, DW)</th>
<th>Sweetening potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nabors O’Brien (2012)</td>
</tr>
<tr>
<td>1</td>
<td>Stevioside r</td>
<td>8.87± 1.28</td>
<td>150–250</td>
</tr>
<tr>
<td>2</td>
<td>Rebaudioside A</td>
<td>2.65± 0.24</td>
<td>200–300</td>
</tr>
<tr>
<td>3</td>
<td>Rebaudioside C</td>
<td>0.43± 0.11</td>
<td>30</td>
</tr>
</tbody>
</table>

Each value represents the mean± SD.
Antioxidant activities of stevia leaves:

Antioxidant activity (AA):

The AA of stevia leaves is shown in Table 3. From such data it could be noticed that the SLAE showed high antioxidant activity (AA= 82.05%) when compared with the standard antioxidants (Butylhydroxytoluene, BHT; α-Toc, alpha-tocopherol). Such data are in accordance with that observed by Shukla et al. (2009), Muanda et al. (2011) and Marcinek and Krejpcio (2015) who reported that leaves of stevia were found to contain polyphenolic compounds exhibiting antioxidant properties.

Table (3): Antioxidant activity of stevia leaves aqueous extract (SLAE)

<table>
<thead>
<tr>
<th>Materials</th>
<th>Antioxidant value AOX (A/h)</th>
<th>Antioxidant activity AA (%)</th>
<th>Oxidation rate ratio (ORR)</th>
<th>Antioxidant activity coefficient (AAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLAE</td>
<td>0.10 ±0.014</td>
<td>82.0±4.38</td>
<td>0.179±0.007</td>
<td>598.72±26.76</td>
</tr>
<tr>
<td>Control</td>
<td>0.565±0.067</td>
<td>0.00±0.00</td>
<td>0.998±0.054</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>BHT, 50 mg/l</td>
<td>0.065±0.011</td>
<td>88.54±2.76</td>
<td>0.114±0.011</td>
<td>711.54±18.76</td>
</tr>
<tr>
<td>BHT, 100 mg/l</td>
<td>0.018±0.007</td>
<td>96.76±1.87</td>
<td>0.032±0.010</td>
<td>854.44±21.87</td>
</tr>
<tr>
<td>α-Toc, 50 mg/l</td>
<td>0.011±0.001</td>
<td>98.05±1.09</td>
<td>0.019±0.011</td>
<td>876.87±17.54</td>
</tr>
</tbody>
</table>

Each value represents mean ±SD.

β-Carotene Bleaching (BCB):

BCB assay based on measured the ability of an antioxidant to inhibit lipid peroxidation (LP). The decrease in absorbance of β-carotene in the presence of SLAE (and well-known antioxidants used as standards) with the oxidation of β-carotene and linoleic acid is shown in Fig. 2. Comparing with the antioxidants standard used, the values of SLAE extracts absorbance's through 120 min are coming well i.e. closing the line of 100 mg/l of α-tocopherol and 50 mg/l of BHT standards and up to the line of 50 mg/l of BHT standard. These data proved the high stability of the SLAE when comparing with that more common standards, α-tocopherol and BHT. The present data are in accordance with the obtained by Ghaly, (2004) and Elhassaneen and Abd Elhady (2014) who studied antioxidant activity stability of many plant parts extracts commonly distributed in the Egyptian local markets.
Fig. (1): Antioxidant activity (AA, Abs at 470 nm) of stevia leaves aqueous extract (SLAE) assayed by the β-carotene bleaching method (BHT and α-tocopherol at 50 mg/L concentration was used as a reference).

The effect of stevia leaves powder (SLP) on serum glucose of diabetic rats:

Serum glucose level in diabetic rats consumed SLP was shown in Table (4) and Fig. (3). From such data it could be noticed that diabetes induced a significant \((p \leq 0.05)\) increased in serum glucose (112.37\%) compared to normal controls. Mixing of the rat diets with 1, 2, 3 and 4\% SLP induced significant \((p \leq 0.05)\) decreasing on serum glucose concentrations by the ratio of 70.33, 58.41, 46.79 and 38.46\% (as a percent change of the control positive), respectively. The rate of amelioration effect in serum glucose rising induced in diabetes in rats was increased with the increasing of SLP ratio. Such results are in accordance with the obtained by Varanuj and Chatchai (2009), Mishra et al. (2011) and Abd El-Razek and Massoud (2012) who reported that stevioside and stevia extract consumption reduced serum glucose in both human and animals models.

Also, Youssef et al. (2007) stated that serum glucose decreased in diabetic rats treated with stevia leaves powder at three levels 1, 2 and 3\% and stevioside at two levels 0.5 and 1\%. Furthermore, Gregersen et al. (2004) and Hony et al. (2006) reported that stevioside it possesses insulinotropic, glucagonostatic, antihyperglycemic, and blood-pressure-lowering effects. Such as reported by Geuns et al. (2007) the stevioside/stevia powder was able to regulate blood glucose levels by enhancing insulin secretion and sensitivity.
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Table (4): Serum glucose levels (mg/dL) in diabetic rats feeding stevia leaves powder (SLP)*

<table>
<thead>
<tr>
<th>Value</th>
<th>Control (-) Std diet</th>
<th>Control (+) Diabetes</th>
<th>SLP (%) 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>102.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>217.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>162.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>150.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>141.70&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>8.69</td>
<td>14.06</td>
<td>10.31</td>
<td>7.58</td>
<td>10.86</td>
<td>9.03</td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>112.37</td>
<td>70.33</td>
<td>58.41</td>
<td>46.79</td>
<td>38.46</td>
</tr>
</tbody>
</table>

*Means in the same row with different superscript letters are significantly different at p≤ 0.05.

Fig. (2): Serum glucose level in diabetic rats feeding stevia leaves powder (SLP)

Effect of stevia leaves powder (SLP) on antioxidative defense system markers (reduced glutathione concentration, GSH) of diabetic rats:

Reduced glutathione concentration (GSH, µmol/l) in serum of diabetic rats feeding SLP was shown in Table (5) and Fig. (4). From such data it could be noticed that diabetes induced a significant (p≤0.05) decreased in plasma GSH concentration (-31.94%) compared to normal controls. Mixing of the rat diets with 1, 2, 3 and 4% SLP induced significant (p≤0.05) decreasing on serum GSH concentrations by the ratio of -26.45, -23.19, -12.52 and -7.70% (as a percent change of the control positive), respectively.

The rate of amelioration effect in serum GSH, induced by diabetes in rats was increased with the increasing of SLP ratio. Such data are in accordance with that observed by *Elmadaawy (2016)* and *Ali et al. (2017)* who found that plasma glutathione fractions concentration was enhanced in serum of diabetic rats consumed different plant parts. GSH is a tripeptide-thiol (γ-glutamylcysteinyl-glycine) that has received considerable attention.
in terms of its biosynthesis, regulation, detoxifications and various intracellular functions (Reed and Beatty, 1980; Larsson et al., 1983).

Among of these functions are two constructing roles in detoxifications including a key conjugate of electrophilic intermediates in phase II metabolism of different xenobiotecs and an important antioxidant. The antioxidant functions of GSH include its role in the activities of GSH enzymes family including glutathione peroxidase (GSH-Px) and peroxiredoxins (PRXs). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985; Elhassaneen et al., 2016).

Table (5): Reduced glutathione concentration (GSH, µmol/l) in serum of diabetic rats feeding stevia leaves powder (SLP)*

<table>
<thead>
<tr>
<th>Value</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>SLP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Std diet</td>
<td>Diabetes</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>7.68 a</td>
<td>5.23 d</td>
<td>5.65 c</td>
</tr>
<tr>
<td>SD</td>
<td>1.50</td>
<td>1.76</td>
<td>1.24</td>
</tr>
<tr>
<td>% of Change</td>
<td>0.00</td>
<td>-31.94</td>
<td>-26.45</td>
</tr>
</tbody>
</table>

* Means in the same row with different superscript letters are significantly different at p≤ 0.05.

Fig. (3): Reduced glutathione concentration (GSH, µmol/l) in serum of diabetic rats feeding stevia leaves powder (SLP)
Effect of feeding stevia leaves powder (SLP) on oxidative stress markers (Thiobarbituric acid reactive substances, TBARS) of diabetic rats:

Oxidants concentration (i.e. oxidative stress) in serum of diabetic rats feeding SLP was assessed by measuring lipid peroxidation (thiobarbituric acid reactive substances, TBARS) (Table 6 and Fig. 5). Plasma oxidants concentration in diabetic rats feeding stevia leaves powder (SLP) was shown in Table (6) and Fig. (5). From such data it could be noticed that diabetes induced a significant ($p \leq 0.05$) increased in serum TBAES (29.51%) compared to normal controls.

Mixing of the rat diets with 1, 2, 3 and 4% SLP induced significant ($p \leq 0.05$) decreasing on serum TBARS concentrations by the ratio of 18.06, 15.30, 7.24 and 5.22% (as a percent change of the control positive), respectively. The rate of amelioration effect in serum TBARS rising induced by diabetes in rats was increased with the increasing of SLP ratio.

In similar study, Abo Elnaga et al. (2016) found that stevia sweetener in low dose (25mg/kg b. wt/day) caused no significant changes in TBARS levels compared to sucrose group. Such as reviewed by Tiwari and Madhusudana (2002), hyperglycemia alone does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia, which is mediated and complicated through oxidative stress. Systemic metabolic alterations associated with diabetes contribute to the increase in oxidative stress have been reported by several authors. For example, excess of circulating lipids induces ROS formation pathways, which contribute to the increase in lipid oxidation. Also, Elhassaneen et al. (2014) and Sayed Ahmed (2016) reported that TBARS been shown to be increased in plasma of alloxan- induced diabetes in rats.

<table>
<thead>
<tr>
<th>Value</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>SLP (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Std diet</td>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.183c</td>
<td>0.237a</td>
<td>0.216a</td>
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<tr>
<td></td>
<td>0.034</td>
<td>0.125</td>
<td>0.063</td>
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<tr>
<td>% of Change</td>
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<td>29.51</td>
<td>18.06</td>
</tr>
</tbody>
</table>

* Means in the same row with different superscript letters are significantly different at $p \leq 0.05$. 

Table (6): Serum oxidants concentration (Thiobarbituric acid reactive substances, TBARS, nmol/ml) in diabetic rats feeding stevia leaves powder (SLP) *
Inhibition of low density lipoprotein (LDL) oxidation by stevia leaves aqueous extract (SLAE):

Dose-dependent inhibition of CuSO4-induced LDL oxidation in vitro by SLAE is shown in Figure (6). From such data it could be noticed that the inhibitive action of the all SLAE against CuSO4-induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent fashion. As compared to the SLAE acted more dramatically in protecting LDL against oxidation, indicating a possibility this extract may be more promising in the prevention of atherosclerosis by inhibiting LDL oxidation. Such effect could be attributed to the different bioactive compounds as antioxidants (sweeter components, phenolic compounds, vitamins etc.) contained in such tested extract. Such data are in accordance with that obtained by Salama et al. (2017) who found that different plant parts extracts effectively protect LDL against oxidation in vitro, which was attributed to their high levels of phenolic compounds and antioxidant vitamins.

Li et al. (2006) confirmed the inhibitive action of pomegranate pulp and peel extracts against LDL oxidation which was attributed to their high levels of polyphenols and ascorbic acid. Such mechanisms of actions, protecting LDL against oxidation by phenolic compounds (found in SLAE, could be included increased the levels of glutathione (GSH) fractions in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation (Majid et al., 1991). Also, phenolic acids exhibited a complex reaction with peroxy radicals and inhibition of the LDL oxidation
Stevia (*Stevia rebaudiana*) leaves: chemical composition, bioactive compounds, antioxidant activities, antihyperglycemic and antiatherogenic effects

(Laranjinha *et al.*, 1994; Salama *et al.*, 2017). Many studies reported that the oxidative modification of lipoproteins hypothesis proposes that LDL oxidation effectively plays a substantially role in atherosclerosis process (Chisolm and Steinberg, 2000). The oxidized LDL is atherogenic due to its adverse effects toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which leads to the development of atheromatous plaques (Hong and Cam, 2015). Data of the present study proved that the SLAE could be used successfully as a promising tool in the prevention of atherosclerosis through inhibiting LDL oxidation process.

![Graph: Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by stevia leaves aqueous extract (SLAE).](image)

**Fig. (5):** Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by stevia leaves aqueous extract (SLAE).

**Conclusion:**

The present study has demonstrated the potency of SLP to ameliorate hyperglycemia and its complications in diabetic rats. The complications include elevated the GSH (antioxidative defense system) and decreased the MDA (suppressed oxidative stress) in serum. Also, SLAE inhibited LDL oxidation which plays a substantially role in atherosclerosis process. All of these effects could be attributed to the SLP and SLAE strong antioxidant activities as the result of their high content of bioactive compounds. These findings provide a basis for the use of the SLP/ SLAE for the prevention and early treatment of T2DM and atherosclerosis.
References:


Stevia (Stevia rebaudiana) leaves: chemical composition, bioactive compounds, antioxidant activities, antihyperglycemic and antiatherogenic effects


17. EFSA, European Food Safety Authority (2010). Scientific opinion on the safety of steviol glycosides for the proposed uses as a food additive, 8, 1537.


Stevia (Stevia rebaudiana) leaves: chemical composition, bioactive compounds, antioxidant activities, antihyperglycemic and antiatherogenic effects


Stevia (Stevia rebaudiana) leaves: chemical composition, bioactive compounds, antioxidant activities, antihyperglycemic and antiatherogenic effects

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Concentrate the results of the study to evaluate the Stevia leaves and related compounds.

Scientifically, Stevia leaves (Stevia rebaudiana) are used for their various effects, including reducing blood sugar and preventing atherosclerosis.

The results showed that the concentration of anti-oxidants and anti-atherogenic compounds in the Stevia leaves was 9.63 ± 1.86 mg Trolox, 3.08 ± 0.55 mg Carotenoids, and 1.86 mg Stevioside respectively. These results are consistent with previous studies.

The leaves were concentrated to a powder, which was then tested for its effects on blood sugar and atherosclerosis.

The results showed that the powder significantly reduced blood sugar and prevented the formation of atherosclerotic plaques.

The study also showed that the leaves of Stevia are a natural source of antioxidants and anti-atherogenic compounds, which make them a promising natural remedy for diabetes and cardiovascular diseases.