Potential Therapeutic Effects Of Stevia Extract On Hepatotoxic Diabetic Rats

Semary, M. A.¹, Yousef, E. H.¹, Abd El-Aziz, A. A.¹, El-Beltagi Alaa, E. B.²

Department of Nutrition and Food Science, Faculty of Home Economics, Minufiya University¹. Department of Food Science and Technology, Faculty of Agriculture, Minufiya University².

Abstract

The effect of aqueous extract of stevia \textit{(Stevia rebaudiana Bertoni)}, as a natural sweetener, on lowering blood glucose and serum lipid profile in hepatotoxic diabetic rats induced by alloxan (150 mg/ kg body weight) and Ccl4 (2ml / kg body weight). Twenty four male rats, weighing 190±20g were divided into 4 groups as follows negative control (normal) and three hepatotoxic diabetic rat groups, 1st group, positive control (hepatotoxic diabetic , untreated), 2nd and 3rd groups (hepatotoxic diabetic animals) were given stevia extract and aspartame orally for 6 weeks at a daily dose of 400 mg/kg body weight. The results indicated that treatments with stevia extract and aspartame significantly \((P \leq 0.05)\) decreased the levels of blood glucose, total cholesterol (TC), Triglycerides (TG), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) as compared with positive control rats. Stevia extract was more effective \((P \leq 0.05)\) in reducing serum TC, TG, LDL-c and VLDL-c than aspartame. On the other hand stevia extract significantly \((P \leq 0.05)\) improved high density lipoprotein cholesterol (HDL-c) compared with positive control. Treatments with stevia extract significantly \((P \leq 0.05)\) increased glutathione- S- transferase (GST) and catalase CAT and significantly \((P \leq 0.05)\) decreased malondialdehyde (MDA) compared to control (+) group, while, there was no significant difference of CAT among positive
and aspartame group. Oral administration of stevia extract and aspartame significantly (P≤0.05) decreased Alanine aminotransferase (ALT), Gamma-Glutamyl Transferase (GGT) and Alkaline phosphatase (ALP) as compared with positive control rats. Oral administration of aspartame cased a significant (P≤0.05) increased in aspartate aminotransferase (AST) compared to positive group. Stevia extract was more effective (P≤0.05) in reducing serum AST, ALT, GGT and ALP than aspartame.

In conclusion, this study indicated that stevia aqueous extract, as a natural sweetener, had hypoglycemic, hypolipidaemic effects as well as improved the antioxidant status of hepatotoxic diabetic rats.

**Keywords:** stevia, aspartame, diabetes, antioxidant, liver disease.
Introduction

Chronic disease constitutes a fast increasing burden to society. The World Health Organization (WHO) estimates that 46% of global disease and 59% of mortality is due to chronic diseases. Thirty-five million individuals in the world die each year from chronic disease and the numbers are increasing steadily. (Bengmark, 2006).

Diabetes mellitus is a complex disorder or more properly described as a malfunction of the pancreas (Adams, 1995). According to the (WHO, 2004), there are approximately 177 million people with diabetes worldwide. The global prevalence of diabetes will go up from 8.6% in 2012 to 9.8% in the year 2030 and the numbers of people affected with diabetes will go up from 285 to 435 million (Neogi, 2007).

The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. According to the Office for National Statistics in the United Kingdom, liver disease is now the fifth most common cause of death after heart disease, stroke, chest disease and cancer. However, unlike other major causes of mortality, liver disease rates are increasing rather than declining (Maton et al., 1993).

Sweeteners are food additives that are used to improve the taste of everyday foods. Artificial sweeteners have increasingly become an area of controversy in the world of food, health and nutrition (Scheufele and Tewksbury, 2007). Aspartame is composed of methyl ester of the dipeptideL-L-aspartyl-L-phenylalanine and a source of 4 Kcal of energy (Food and Drug Administration, 2006). It is the most used artificial sweetener in the world and it used in different food products as a food additive (Mazur, 1984).

Stevia a natural sweetener plant having medicinal and commercial importance is being used all over the world (Ahmed et al., 2007). Leaves of this plant have 30-45 times the sweetness of sucrose. The plant has long history of its medicinal use in Paraguay and Brazil (Prakash et al., 2008). Natural sweeteners as compared to artificial sweeteners are thought to be safe because their extracts are derived from plants. However artificial sweeteners are compounds that have very little
or no nutritional value (American Dietetic Association, 2004). Therefore, the present study was aimed to evaluate the potential therapeutic effects of stevia extract on hepatotoxic diabetic rats.

Materials And Methods

Materials:
Casein, cellulose, choline chloride, DL-methionine, vitamins mixture and minerals mixture were obtained from Morgan chemical Co. Cairo, Egypt. Alloxan and Carbon tetrachloride (Ccl₄) was obtained from El-Gomhoryia Company for Drug, Chemicals and Medical instruments, Cairo, Egypt. Stevia leaves (Stevia rebaudiana Bertoni) were piked from the Agriculture Research Center farm, Giza, Egypt. Aspartame was obtained from the local market, shebin Elkom.

Methods:
Preparation of stevia aqueous extracts:
Dried stevia leaves were extracted by using water according to Kinghorn et al. (1984). Hot water 80º± 2 was added to dried leaves (5g leaves: 95ml water) and left for 7 h, then filtered through Whatman No. 4 filter paper.

Biological investigation:
Induction of diabetes and hepatotoxicity in experimental animals
Diabetes was induced in normal healthy adult male rats by intraperitoneal injection of alloxan 150mg\kg body weight once according to the method described by (Desai and Bhide, 1985). Six hours after the injection of alloxan, fasting blood samples were obtained by retro-orbital method to estimate fasting serum glucose. Rats having fasting serum glucose more than 200 mg/dl were considered diabetics. Diabetic rats were treated subcutaneous injection (0.5 ml of 1:1 mixture of Ccl₄and olive oil) based on a calculated (2ml / kg b. wt.) twice a week for two weeks to induce chronic damage of the liver according to the method described by Jayasekhar et al., (1997). After the injection of CCl₄, blood samples were obtained by retro orbital method to ensure occurrence of liver injury through estimate liver function.

Experimental design
The experiment were done at the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. Rats were housed in wire cages in a room maintained at 25 ± 20ºc and kept under
normal healthy conditions. All rats were fed on standard diet for one week for adaptation then rats were divided into four equal groups (6 rats each). Group 1: Negative control group (normal), the other three groups were hepatotoxic diabetic rats, the first hepatotoxic diabetic group, positive control group (hepatotoxic diabetic rats), the second and the third hepatotoxic diabetic groups were given stevia aqueous extract and aspartame at dose 400mg/kg/day for 42 days. Experimental diet prepared from basal diet by ratio of 12, 10, 4, 1, 4, 10, 0.2, 0.3, 58.5% for Protein, Corn oil, Mineral Mixture, Vitamin mixture, Cellulose, Sucrose, Choline chloride, Methionine and Corn starch respectively according to AIN, (1993). The composition of salt mixture (mg / kg salts) according to Hegested et al., (1941) were 600, 645, 150, 204, 334, 55, 1.6, 10, 0.5 and 0.06 mg/kg salts for CaCO3, K2 HPO4, Ca HPO4. 2H2O, MgSO4.2H2O, Nacl, Fe (C6H5O7) 26H2O, KI, MnSO4.4H2O, Zncl2 and Cu SO4. 5H2O respectively. The composition of vitamin mixture (mg / kg) according to Campbell, (1963) were 10 I.U., 0.50 I.U, 200 I.U., 0.50 mg, 1.00 mg, 4.00 mg, 0.40 mg, 100 I.U., 200 mg, 0.02 mg, 24 mg, 0.02 mg, 2.00 mg and 0.02 mg for Vitamin E, Vitamin K, Vitamin A, Thiamin, Pyridoxine, Niacin, Calcium panthothenic acid, Vitamin D, Choline chloride, Folic acid, Inositol, Para-amino – benzoic acid, Vitamin B12 and Biotin respectively. During the experimental period, all rats were weighed and the consumed diets were recorded every day. At the end of the experiment rats were fasted over night (12 hours) and anesthetized with diethyl ether. Blood samples were collected into a dry clean centrifuge glass tubes. Serum was separated by centrifugation at 4000 r.p.m for 15 minutes at room temperature (220° C). Serum was carefully aspirated and transferred into clean quiet fit plastic tubes and kept frozen at (-20° C) until analysis. Blood sample were collected in ethylene diamine tertra acetic acid (EDTA) as anticoagulant for determination CAT, GST and MDA. The organs (liver, kidney, heart, spleen, lungs, brain) were removed and washed in saline solution and weighed. Biological evaluation of the experimental diets was carried by determination of initial weight, final weight, body weight gain (g) and feed intake according to Chapman et al., (1959) using the following formulas.

\[ \text{BWG} = \text{Final weight} - \text{Initial weight} \]
Relative organ weight (%) = \frac{\text{Organ weight}}{\text{body weight}} \times 100

**Biochemical analysis:**

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the methods described by Yound (1975). Alkaline phosphatase (ALP) and gamma-Glutamyl Transferase (GGT) were determined according to the methods described by IFCC methods., (1983) Gowenlock et al., (1988) respectively. Total protein and albumin and were determined according to the methods described by Nils, (1983). Serum total bilirubin was determined according to the methods described by Doumas et al., (1973). Triglycerides was carried out according to Fassati and PrencipeL (1982). Total cholesterol was determined by colorimetric method according to Allain (1974). Determination of high density lipoprotein (HDL-c) was carried out according to the method of Fnedewaid (1972) and Gordon and Amer (1977). Very low density lipoproteins (VLDL-c) and low density lipoproteins (LDL-c) were determined according to Lee and Nieman (1996) being calculated as follows:

\[
\text{VLDL-c (mg / dl)} = \frac{\text{triglycerides}}{5} \\
\text{LDL-c (mg / dl)} = \text{total cholesterol} - (\text{HDL-c} + \text{VLDL-c}).
\]

Malondialdehyde, glutathione- S- transferase and catalase activity were determined according to Ohkawa et al., (1979), Habig et al., (1974) and Aebi (1984), respectively. Creatinine and uric acid were determined according to the method described by Henry, (1974) and While et al., (1970) respectively. Serum glucose was determined using chemical kits according to Trinder (1969).

**Statistical Analysis:**

The data were analyzed using a completely randomized factorial design SAS, (1988), when a significant main effect was detected, the means were separated with the student-Newman- Keuls test. Differences between treatments at \( p \leq 0.05 \) were considered significant.

**Results And Discussion**

The effect of stevia and aspartame on serum glucose of normal and hepatotoxic diabetic rats shows in Table (1). There was no significant \( (P>0.05) \) difference in blood glucose for negative group along the period of experiment (table 1). As well as, there was no \( (P>0.05) \) significant difference in blood glucose between positive and aspartame groups after 3 weeks. On the other side, there was a significant \( (P>0.05) \) decreased in blood glucose in stevia group compared to positive group after 3 weeks of oral administration by ratio of 8.11%. On the other side,
administration of stevia and aspartame caused significant (P≤0.05) decrease in blood glucose level at the end of experiment compared to zero and 3 weeks by ratio of 20.58 and 13.88% respectively.

Alloxan is a specific toxin that causes massive destruction of the pancreatic B-cells, provoking a state of primary deficiency of insulin without affecting other islet types, and thus creating a hyperglycaemic condition (Aruna et al., 1999).

After 6 weeks the administration of hepatotoxic diabetic rats with stevia and aspartame caused a significant (P≤0.05) decrease in blood glucose level compared to positive group by ratio of 28.95 and 18.42% respectively.

According to the previous results the effect of different solutions potency on fasting serum glucose compared to (+) control group have the following sequences: stevia > aspartame.

These results agree with Megeji et al., (2005) who reported that stevia leaf extract has been used traditionally in the treatment of diabetes, where a single acute dose of stevioside (1000 mg) was able to reduce blood glucose.

Several human trials conducted on normal healthy volunteers have shown that extracts of stevia rebaudiana leaves could increase glucose tolerance in humans. Therefore stevia may be advantageous in the treatment of type 2 diabetes (Gregersen et al., 2004 ; Chen et al., 2008 and Anton et al., 2010). The major components of the stevia leaves are stevioside, rebaudioside A, rebaudioside and dulcoside A (Wood et al., 1955). Stevioside, steviol and rebaudioside A stimulate glucose uptake by increasing insulin secretion from β cell of pancreas and enhancing insulin sensitivity of peripheral tissues promoting glucose uptake. Therefore, they exhibit anti hyperglycemic action by reducing glucose production while increasing glucose uptake to maintain plasma glucose balance (Varanuj and Chatchai, 2009). These results in dis agreement with Prokić et al., (2014) indicated that serum concentrations of glucose increased during aspartame treatment.

Effect of stevia and aspartame on liver functions of normal and hepatotoxic diabetic rats summarized in Table (2). Hepatotoxic diabetic rats showed an elevation in AST, ALT, GGT and total billurubin compared to control negative group. While, there were significant (P≤0.05) decrease in total protein and albumin compared to control negative group at zero time. After six weeks administration of stevia and aspartame lead to a significant (P≤0.05) decrease in ALT, GGT, ALP and total bilurubin as compared with positive group. Oral administration of stevia for 6 weeks caused a significant (P≤0.05) decreased in AST compared to positive group by ratio of 34.78%. On the other side, there
was a significant ($P \leq 0.05$) increased in AST in aspartame group compared to positive group by ratio of 27.93%. The highest decreased in ALT, GGT and ALP activities belonged to rats administrated with stevia extract by ratio of 33.76, 16.12 and 24.5% respectively.

According to the previous results the effect of different solutions potency on serum AST, ALT, GGT, ALP, TP and T. Bill compared to (+) control group have the following sequences: stevia > aspartame.

In similar studies Abdelsattarelbatran et al., (2006) reported that stevia leaves powder and its polyphenol extract caused a significant decrease in the level of ALT and AST of serum and had protective effect on the liver damage of diabetic treated rats. Also, Shaheen and Afifi (2014) found that administration of aspartame at a dose level of 500 mg/kg b.wt to rats for 42 days significantly elevated the levels of serum (ALT), (AST), (GGT), tumor necrosis factor (TNF-α) and hepatic alpha fetoprotein (AFP) activity which indicated injury to the liver functions.

There were significant ($P \leq 0.05$) increases in total protein and albumin between stevia and aspartame groups compared to positive group by ratio of 73.33 and 33.33% for total protein and 177.5 and 154.16 % for albumin respectively.

An increase in the level of plasma total protein, decrease in albumin with significant increase in total bilirubin observed in aspartame treated rats by (Choudhary and Devi, 2014).

Data presented in Table (3) illustrated the effect of stevia and aspartame on total cholesterol, triglyceride, HDL-c, LDL-c and VLDL-c of normal and hepatotoxic diabetic rats. The results showed that positive group had higher significant levels of ($P \leq 0.05$) TC, TG, LDL-c and VLDL-c than negative control rats while, HDL-c value had an opposite trend. The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles (Mooradian, 2009).

According to the previous results the effect of different solutions potency on serum lipid profile including TC, TG, HDL-c, LDL-c and VLDL-c compared to (+) control group have the following sequences: stevia > aspartame.

High levels of triglycerides and LDL-c are associated with high risk of coronary dysfunction, whereas increase in HDL-c is associated with decrease in coronary risk (Alam et al., 2013).

The main cause of the lipid changes associated with diabetic dyslipidemia is the increased free fatty-acid release from insulin-resistant fat cells (Solano and Goldberg, 2005 and Chahil and Ginsberg, 2006).
Administration of rats with stevia and aspartame significantly (P≤0.05) reduced TC, TG and VLDL-c as compared with positive control rats. There was no significant difference for LDL-c between positive and aspartame groups. Regarding to HDL-c there was significant increased in stevia group compared to positive group by ratio of 44.78% while, there was no significant difference in HDL-c among positive and aspartame groups. Stevia was more significantly effective (P≤0.05) in improvement of serum TC, TG, LDL-c, VLDL-c and HDL-c than aspartame group by ratio of 16.39, 33.04, 28.08, and 44.78% respectively. However, these values are still significantly higher (P≤0.05) than those values of negative control rats.

These results are in agreement with that obtained by Sharma et al., (2007) and Sharma et al., (2009), who reported that the consumption of stevia extract reduced the levels of cholesterol, triglyceride, LDL-c significantly and increased the levels of HDL-c.

Administration rats of aspartame at a dose of 500 mg/kg b.wt. to rats for 42 days significantly increased, total cholesterol, triglycerides and low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) are reported by Shaheen and Afifi (2014).

Effect of stevia and aspartame on serum kidney functions of normal and hepatotoxic diabetic rats summarized in table (4). There are no significant difference in creatinine values between negative and stevia groups. On the other side, there was a significant decreased in creatinine in stevia and aspartame groups compared to positive group. The value of uric acid was significantly (p≤ 0.05) increased in positive, stevia and aspartame groups compared to negative group. Oral administration of stevia caused a significant decreased in uric acid compared to positive group while, no significant difference observed in uric acid positive and aspartame groups.

In similar study Shaheen and Afifi (2014) found that oral administration of aspartame (500mg/kg.b.wt.) for 42 days significantly increased serum urea and creatinine.

Data presented in table (5) illustrated effect of stevia and aspartame on GST, CAT and MDA of normal and hepatotoxic diabetic rats. There were a significant (P≤0.05) decreasing in the levels of glutathione-S-transferase (GST) and catalase (CAT) in positive group compared to negative group while, MDA had an opposite trend by ratio of 38.98, 38.64% respectively.

Administration of stevia and aspartame significantly (P≤0.05) increased GST compared to positive group by ratio of 35.63 and 10.51% respectively. On the other side, there was significant (P≤0.05) decrease in CAT for aspartame group compared to positive groups by ratio of 1.82%. Stevia was more effective (P≤0.05) in improvement of GST and
CAT by ratio of 35.63 and 44.45% respectively. Administration of stevia and aspartame induced a significantly (P≤0.05) decreased in the MDA compared to positive group by ratio of 28.23 and 10.29% respectively. The highest reduction (P≤0.05) for MDA was observed in stevia group.

According to the previous results the effect of different tested plant parts potency on GST, CAT and MDA compared to (+) control group have the following sequences: stevia > aspartame.

In similar study carried out by Mourad, (2011) found that (GSH) content was significantly increased in the liver tissue after 2, 4 and 6 weeks of aspartame administration. However CAT activity significantly decreased in the liver tissue after 2 and 4 weeks of aspartame administration.

Administration of aspartame at a dose level of 500 mg/kg b.wt. to rats for 42 days significantly decreased liver glutathione (GSH) are reported by Shaheen and Afifi, (2014).

Yadav et al., (2012) showed that stevia extract possessed significant increasing of catalase activity (p<0.01), and decreasing in malondialdehyde level (p<0.001) present in liver tissues.

The daily oral administration of aspartame (40 mg/kg) for 2, 4 and 6 weeks caused a significant increase in MDA levels determined in the liver tissue after 4 and 6 weeks (Mourad, 2011).

These result disagree with that of Maté, (2000) who reported that with During the Aspartame treatment, the activity of CAT increased, while the activity of SOD decreased, but that decrease was not statistically significant.

Data presented in table (6) show the effect of stevia and aspartame on FI, BWG and FER of normal and hepatotoxic diabetic rats. The results showed that positive group had significantly lower (P≤0.05) FI, BWG and FER than negative group. There were no significant (P>0.05) differences in FI in rats administrated with stevia, aspartame and negative group. The BWG in rats administrated with stevia and aspartame was significantly (P≤0.05) lower than negative control group. The lowest BWG was observed in aspartame group. On the other hand, there was significant (P≤0.05) increases in FER of stevia and aspartame groups compared to control positive group.

According to the previous results the effect of different tested plant parts potency on FI and BWG compared to (+) control group have the following sequences: stevia > aspartame.

These results agree with Gregersen et al., (2004) who suggested that stevia may have a role in food intake regulation. Also Dela Hunty et al., (2006) indicated that aspartame reduces food intake and may assist with weight control. On the other hand, these results differed from those obtained by Blundell and Hill (1986) and Swithers and Davidson
(2008) who suggested that aspartame may paradoxically stimulate appetite and thereby lead to weight gain.

The effect of stevia and aspartame on relative organs weight (%) of normal and hepatotoxic diabetic rats are shown in table (7). No significant (P>0.05) differences in relative heart weight was observed among positive, stevia and aspartame groups. Positive control groups had higher relative (P≤0.05) liver and kidney weights than all groups. Sabu and Kuttan (2002) reported that alloxan effecting organs such as liver, kidney, pancreas and haemopoitic system.

Kidney enlargement is an early feature of diabetes due to an increase in the capillary length and diameter, and was correlated with the degree of glycemic control (Melis, 1995; Melis, 1996 and Melis, 1999).

Negative control and stevia groups had significant lower relative (P≤0.05) liver, kidney, lungs and spleen weights than positive group. However aspartame group was non significantly lower relative heart weight compared to negative and stevia groups. The study carried out by Parthasarathy et al., (2006) reported that after 30 and 90 days aspartame administered animals showed a marked decrease in organ weight / animal weight ratio.

Relative lungs weight significantly (P≤0.05) increased in positive control, stevia and aspartame groups compared to negative group. Aspartame had a higher (P≤0.05) relative lungs weight than stevia group.

References


Yadav, R.; Manivannan, E. and Sharma, R. (2012): Study of effect of stevia rebaudiana bertoni extract on oxidative stress in Type-2 diabetic rat models. 3rd World Congress on Diabetes & Metabolism, September 24-26, Marriott Convention Center, Hyderabad, India


Table (1): Effect of stevia and aspartame on serum glucose of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (week)</th>
<th>Negative group</th>
<th>Hepatotoxic diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Serum Glucose (mg/dl)</td>
<td>97.66±1.98</td>
<td></td>
<td>178.5±1.61</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>185±4.47</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>190±8.94</td>
</tr>
</tbody>
</table>
Each value in the table is the means ± SD. Small letters (a, b, c, d) in the same column significantly different (P ≤0.05) among experimental periods. Capital letters (A, B, C, D) in the same row significantly different (P ≤0.05) among groups. Mixture: stevia, fructose, sodium glyconate and β-galactosidase enzyme.

Table (2): Effect of stevia and aspartame on liver functions of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Negative group</th>
<th>Hepatotoxic diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>AST (U/L) I</td>
<td>51b±2.37</td>
<td>90.7a±3.66</td>
</tr>
<tr>
<td>F</td>
<td>52.5a±2.05</td>
<td>92b±1.78</td>
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<tr>
<td>ALT (U/L) I</td>
<td>44b±3.58</td>
<td>74.5a±4.84</td>
</tr>
<tr>
<td>F</td>
<td>42c±4.73</td>
<td>77a±3.22</td>
</tr>
<tr>
<td>GGT (U/L) I</td>
<td>40.33b±3.22</td>
<td>62.5a±1.94</td>
</tr>
<tr>
<td>F</td>
<td>40.00d±3.22</td>
<td>62.0a±2.68</td>
</tr>
<tr>
<td>ALP (U/L) I</td>
<td>54b±4.09</td>
<td>73a±2.37</td>
</tr>
<tr>
<td>F</td>
<td>54.33d±1.37</td>
<td>80a±2.68</td>
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<tr>
<td>TP (mg/dl) I</td>
<td>5.5a±0.49</td>
<td>2.8b±0.23</td>
</tr>
<tr>
<td>F</td>
<td>6a±0.64</td>
<td>3d±0.56</td>
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<tr>
<td>Alb (mg/dl) I</td>
<td>3.8a±0.18</td>
<td>1b±0.36</td>
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<tr>
<td>F</td>
<td>4a±0.45</td>
<td>1.2a±0.18</td>
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<tr>
<td>T. Bill (mg/dl) I</td>
<td>0.21b±0.008</td>
<td>1a±0.18</td>
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<tr>
<td>F</td>
<td>0.23c±0.22</td>
<td>1.2a±0.36</td>
</tr>
</tbody>
</table>

Each value in the table is the means ± SD. Different letters (a, b, c, d) in the same row significantly different (P ≤0.05). Mixture: stevia, fructose, sodium glyconate and β-galactosidase enzyme. I : Initial, F : Final, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-Glutamyl Transferase, ALP: Alkaline phosphatase, TP: Total protein, Alb: albumin and T. Bill: Total bilirubin.

Table (3): Effect of stevia and aspartame on lipid profile of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Negative group</th>
<th>Hepatotoxic diabetic groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>125d±4.47</td>
<td>199.33a±4.49</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>60d±2.37</td>
<td>110a±4.47</td>
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### Table (4): Effect of stevia and aspartame on GST, CAT and MDA of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
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<th>Hepatotoxic diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST (U/mg)</td>
<td></td>
<td>304±3.2</td>
<td>185.5±3.4</td>
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<tr>
<td></td>
<td></td>
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<td>251.6±3.7</td>
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<td>205±4.5</td>
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<tr>
<td>CAT (U/mg)</td>
<td></td>
<td>110.9±0.9</td>
<td>68.05±1.9</td>
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<tr>
<td></td>
<td></td>
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<td>98.3±1.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>69.29±1.4</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td></td>
<td>21.6±1.5</td>
<td>42.8±1.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>30.4±2.2</td>
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<td></td>
<td>38±2.4</td>
</tr>
</tbody>
</table>

Each value in the table is the means ± SD. Different letters (a, b, c, d) in the same row significantly different (P ≤0.05). **Mixture:** stevia, fructose, sodium glyconate and β-galactosidase enzyme. **TC:** Total cholesterol, **TG:** Triglycerides, **HDL-c:** high density lipoprotein cholesterol, **LDL-c:** low density lipoprotein cholesterol and **VLDL-c:** Very low density lipoprotein cholesterol.

### Table (5): Effect of stevia and aspartame on serum kidney functions of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Negative group</th>
<th>Hepatotoxic diabetic groups</th>
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<tbody>
<tr>
<td>Creatinine</td>
<td></td>
<td>0.45±0.04</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.48±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>Parameter</td>
<td>Uric Acid (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2(^c) ± 0.27</td>
<td>3.5(^a) ± 0.45</td>
<td>2.9(^b) ± 0.32</td>
</tr>
</tbody>
</table>

Each value in the table is the means ± SD. Different letters (a, b, c, d) in the same row significantly different (P ≤0.05). **Mixture:** stevia, fructose, sodium glyconate and β-galactosidase enzyme.

**Table (6):** Effect of stevia and aspartame on FI, BWG and FER of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Negative group</th>
<th>Hebatotoxic diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>FI (g/42d)</td>
<td>16.54(^a) ± 0.68</td>
<td>8.64(^b) ± 1.35</td>
</tr>
<tr>
<td>BWG (g)</td>
<td>37.34(^a) ± 1.87</td>
<td>-55(^d) ± 2.53</td>
</tr>
<tr>
<td>FER</td>
<td>0.045(^a) ± 0.001</td>
<td>-1.52(^b) ± 0.09</td>
</tr>
</tbody>
</table>

Each value in the table is the means ± SD. Different letters (a, b, c, d) in the same row significantly different (P ≤0.05). **Mixture:** stevia, fructose, sodium glyconate and β-galactosidase enzyme. **FI:** Feed intake, **BWG:** Body weight gainand **FER:** Feed efficiency ratio.

**Table (7):** Effect of stevia and aspartame on relative organs weight (%) of normal and hepatotoxic diabetic rats

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Negative group</th>
<th>Hepatotoxic diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Heart</td>
<td>0.29(^bc) ± 0.03</td>
<td>0.32(^ab) ± 0.02</td>
</tr>
<tr>
<td></td>
<td>2.36c±0.11</td>
<td>3.55b±0.20</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>kidneys</td>
<td>0.71c ±0.03</td>
<td>1.11b±0.06</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.23c±0.02</td>
<td>0.28b±0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.56b±0.04</td>
<td>0.68a±0.02</td>
</tr>
</tbody>
</table>

Each value in the table is the means ± SD. Different letters (a, b, c, d) in the same row significantly different (P ≤0.05). **Mixture**: stevia, fructose, sodium glycoconate and β-galactosidase enzyme.
التأثيرات العلاجية المحتملة لمستخلص الأستفيا كمحلى طبيعي على الفئران المصابه بالسكر والكبد معاً

آيات محمد سمرى1، هبه عز الدين يوسف1، عبير أحمد عبدالعزيز1، علاء الدين البلتاجى2
قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي - جامعة المنوفية1، قسم علوم وتقنية الأغذية، كلية الزراعة - جامعة المنوفية2

أجريت الدراسة الحالية بهدف استكشاف تأثير المستخلص المائي لـ Stevia (rebaudiana Bertoni) كمحلى طبيعي على خفض سكر الدم ومستويات الدهون في الفئران المصابه بالسكر والكبد معاً. تم الأساتذة بعد 24 فار تقييمهم إلى أربعة مجموعات (6 فار لكل مجموعه) على النحو التالي: المجموعة الضابطه السالبه (فئران غير مصابه بالسكر والكبد معاً)، المجموعتين الأخريين مصابو السكر والكبد معاً، المجموعة الأولى هي عبارة عن المجموعة الضابطه الموجبه (مصابو السكر والكبد معاً) والمجموعة الثانية عبارة عن الفئران مصابو السكر والكبد معاً ولكن تم تم تغذيتهم لمدة 42 يوم على كل من مستخلص الأستفيا والأسبرتام بجرعه يوميه 400 ملجم / كجم من وزن الجسم. وقد أوضحت النتائج أن التغذية على المستخلص المائي لـ Stevia أظهرت النتائج أن التغذية على مستخلص الأستفيا كان أكثر كفاءه (P≤0.05) في كل من VLDL-c و الكوليسترول عالى الكثافه (HDL-c) مقارنة بالمجموعة الضابطه الموجبه. وأظهرت النتائج أن الدراسه أن المستخلص المائي لـ Stevia له تأثير على خفض سكر الدم،تحسين دهون الدم كما يعمل على تحسين مضادات الأكسدة في الفئران المصابه بالسكر والكبد معاً.
الكلمات الكاشفة: استفيا، أسبتام، السكر، الكبد، مضادات الأكسدة