



Journal of Home Economics

Volume 25, Number (4), 2015

<http://homeEcon.menofia.edu.eg>

Journal of Home
Economics

ISSN 1110-2578

Effects Of Stevia (*Stevia Rebaudianabertoni*) And Debettered Stevia Extracts On Hyperglycemic With Hepatotoxicity In Rats.

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Abstract

Stevia, a natural sweetener plant having medicinal and commercial importance is being used all over the world. This study aimed to evaluate the effect of stevia and stevia after remove its bitterness to ameliorate the adverse effects hyperglycemic with hepatotoxic rats. Different β -galactosidase enzyme concentrations 1, 2, 3, 4 and 5% were added to stevia aqueous extract to remove its bitterness. Stevia leaves extract treated by 5% β -glycosidase enzyme was the best treatment. Instant coffee was prepared by added table sugar, dried stevia aqueous extract (DSAE), dried debettered steviaaqueous extract by β -galactosidase enzyme (DDSAE) and aspartame then sensory evaluation showed DDSAE was improve sweetness, bitterness and overall acceptability compared other additives. Forty adult rats were randomly divided into two main groups, the first group, negative control group (n=8), and the second group: hepatotoxic diabetic groups (n=32) fed standard diet and were divided into four sub groups (8 rats/each), subgroup1: positive control group and sub group 2,3 and 4 received a daily oral dose of 400 mg/kg B.W/ day of dried DSAE, DDSAE and aspartame respectively. The results indicated that rats treated with DSAE and DDSAE were significant ($P \leq 0.05$) decreased in the level of blood glucose, aspartate

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T.Bill), improved antioxidant status and weights gain, but DSAE were more effective. In conclusion, the treatment with stevia had the ability to decreasing of blood glucose, lipid oxidation and improving liver functions of hepatotoxic diabetic rats

Key words: Stevia, - debettered stevia- hepatotoxic diabetic rats- instant coffee

Introduction

Sweeteners are food additives that are used to improve the taste of everyday foods (**Scheufele and Tewksbury, 2007**). Table sugar has been an essential component of human diet. Its excess consumption can lead to unhealthy effect on the body, most notably diabetes mellitus. Therefore, sugar substitutes were introduced as safer alternatives. These are now used by millions of people worldwide without knowing their harmful effects on the body (**Tandel, 2011**). Artificial sweeteners have increasingly become an area of controversy in the world of food, health and nutrition (**Scheufele and Tewksbury, 2007**). Aspartame, a non-caloric artificial sweetener, can lead to seizures, headaches and attention deficit disorders. Some people experience heart palpitations, constipation and swelling of certain body parts after consuming this artificial sweetener (**Gardana et al., 2003**). Natural sweeteners are sweet tasting compounds extracted from plants or natural products with some nutritional value.

Stevia (*Stevia rebaudiana*) is an herbaceous perennial shrub indigenous to Paraguay and Brazil. Stevioside, the main sweet component in the leaves of this plant, is approximately 300 times sweeter tasting than sucrose (**Geuns, 2003**). The Stevia leaves have sensory and functional properties superior to those of many other high-potency sweeteners and is likely to become a major source of natural sweetener for the growing food market (**Goyal and Goyal, 2010**). It is commercially well known to exert beneficial effects on human health and has become an interesting area of research these days. Leaves of stevia has many medical applications like anti-hyperglycaemic (**Jeppesen et al., 2003; Benford et al., 2006**) and hepatoprotective (**Mohan and Robert, 2009**). Chronic diseases constitute 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 (**Bengmark, 2006**). Diabetes mellitus (DM) being a chronic metabolic disorder, is characterized by hyperglycemia and disturbances in the metabolism of carbohydrates, lipids and proteins. Apart from hyperglycemia, increased oxidative stress is also reported to play a major role in the pathogenesis of this disease. Oxidative stress occurs due to the glucose autoxidation and glycation of proteins, which thereby depletes the antioxidant defence system and thus promotes free radical generation (**Singh et al., 2013**). The relation between the diabetes and liver disease can be in three different ways 1) liver disease causing diabetes 2) diabetes contributing or causing liver disease and 3) risk factors for liver disease and diabetes are similar (**Baig et al., 2001**). Diabetes mellitus and advanced liver disease are associated with each other more frequently, and such an association carries a significant risk of morbidity and mortality. A metabolic pathway leading to advanced liver disease via fatty liver and steatohepatitis has been demonstrated, further supporting the possibility that cirrhosis may be a late complication of diabetes (**Moscatiello et al., 2007**). The present study was taken up to assess the antidiabetic, antihepatotoxic and antioxidative properties of stevia and stavia which remove its bitterness extracts which help in managing diabetes and hepatotoxicity in adult rats.

Materials and methods

Materials

Stevia leaves (*Stevia rebaudiana* Bertoni) were picked from the Agriculture Research Center farm, Giza, Egypt. Aspartame and chickpea (*Cicer arietinum*) were obtained from the local market of Shebin El-kom, Menofia Government. Alloxan and carbon tetrachloride (CCl₄) was obtained from El-Gomhoryia Company for Drugs, Chemical and medical Industries Trading, Cairo, Egypt.

Methods

Preparation of stevia aqueous extracts:

Dried stevia leaves were extracted by using distilled water according to **Kinghorn et al., (1984)**. Hot water (80°C ± 2) was added to dried leaves (5gm leaves : 95ml water) in 250 ml Erlenmeyer flask,

remained for 7 h then filtered through filter paper whatman 102. The stevia aqueous extract prepared for enzyme treatment was carried out by soaking 5gm of dried stevia leaves in 0.1 M citrate buffer at pH 5 and kept for 7 hr then filtered through Whitman No. 4.

Preparation of β -galactosidase enzyme

Germination of chickpeas

The seeds were sterilized by soaking in 75% ethanol for 1 min. The seeds were soaked in tap water for 12h at room temperature ($\sim 25^{\circ}\text{C}$). The soaked seeds were kept between thick layers of cotton cloth and allowed to germinate in dark at room temperature for 3 days. The germinated seeds were rinsed with tap water and then, ground (**Mansour and Khalil, 1998**).

Extraction and partial purification of β -galactosidase enzyme:

The ground germinated (100g) were homogenized for 60 min at 4°C with 500ml acetate buffer (at optimum pH for β - galactosidase extract) containing 0.5% glycerol. The homogenate was filtered and centrifuged (Jouan, MR, 18-12, France) at 5000 g for 20 min and supernatant referred to as crude extract. Ammonium sulphate concentration (30-70%) was gradually added to the supernatant and then re-centrifuged at 5000g for 20 min. The obtained precipitate was dissolved in 100 ml of water.

Enzyme activity assay

The activity of β -galactosidase was assayed as described by **Li et al., (1975)** using O- nitrophenyl- β -D-galactopyranoside (ONPG) as a substrate. The reaction mixture consisted of 0.2 ml of ONPG, 0.7 ml of 0.02M acetate buffer (pH 5) and 0.1 ml of β -galactosidase extract. The reaction mixture was incubated at optimum temperature for 30 min. The reaction was terminated by the addition of 0.5 ml of 1M sodium carbonate. The resulted O-nitrophenol was estimated spectro-photometrically (UNICO 2802 C/PCS Series spectrophotometer, USA) at 410 nm. One unite of β -galactosidase activity is defined as the amount of enzyme required to release $1\mu\text{mol}$ of o-nitrophenol (ONP) per min under the condition described above.

Debittering of stevia extract by β -galactosidase enzyme treatment:

Different concentrations 1, 2, 3, 4 and 5% of β -galactosidase enzyme (408.36 unit/ml) were added to 100 ml stevia aqueous extract. The mixture

was incubated at 40°C for 1 hr, and then filtered by filter paper whatman No, 102.

Sensory evaluation of crude and debittered stevia extracts

Crude stevia aqueous extract and stevia aqueous extract treated by different concentrations of β -galactosidase enzyme were evaluated for sweet, bitter, metal, sour tastes and general acceptability according to **DuBois and Stephenson, (1985)**.

Freeze-drying aqueous extracts

The crude stevia aqueous extract and stevia aqueous extract treated with 5% of β -galactosidase enzyme were freeze-dried for 24 hr with vacuum freeze dryer tech support (KVCZ 300, V 220 and HZ 50, Kory) and then kept in dark glasses at 5 ± 2 °C till used.

Bioactive compounds of dried stevia extracts:

The total phenolic and total flavonoids content of dried stevia extract and dried debittered stevia extract were determined according to **Wolfe et al., (2003)** and **Chang et al., (2002)** respectively. Total phenolic and total flavonoids content were determined as mg of gallic acid equivalent (GAE) and mg of quercetin (QE)/gram samples respectively.

Biological investigation

Experimental animals

Forty adult male albino rats, Sprague Dawley strain, weighting (200g \pm 5) were used. The animals were derived from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. The rats were housed in wire cage under controlled condition. The diet was introduced to rats in special food container to avoid scattering of food and contamination. Tap water were provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. 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)**. Diabetic rats were treated subcutaneous injection (0.5 ml of 1:1 mixture of CCl₄ and olive oil) based on a calculated (2ml / kg B.W) twice a week for two weeks to induce chronic damage of the liver according to the method described by **Jayasekharet al., (1997)**.

Experimental design

All rats were fed on standard diet for one week for adaptation then rats were randomly divided into two main groups. The first group, negative control group (n=8), and the second group: hepatotoxic diabetic groups (n=32) fed standard diet and were divided into four sub groups (8 rats per each), subgroup 1: positive control group and sub group 2, 3 and 4 received a daily oral dose of 400mg/kg B.W/ day of dried DSAE, DDSAE and aspartame, respectively. At the end of the experiment, rats were fasted overnight (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 rpm for 15 minutes at room temperature. Serum was carefully aspirated and transferred into clean quiet fit plastic tubes and kept frozen at (-20°C) until analysis. Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) as anticoagulant for determination CAT, GST and MDA.

Biochemical analysis

Serum glucose was determined using chemical kits according to **Trinder, (1969)**. Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzymes activities were measured according to the methods described by **Bergmeyer and Harder (1986)**, **Kachmar and Moss (1976)** and **Varley et al., (1980)**, respectively. Gamma-Glutamyl Transferase (GGT) were determined according to the methods described by methods of **Gowenlock et al., (1988)**. Total protein and albumin were determined according to the methods described by **Nils (1983)** and **Rebecca (2006)**. Serum total bilirubin was determined according to the methods described by **Doumaset al., (1973)**. Malondialdehyde (MDA), glutathione-S-transferase (GST) and catalase (CAT) activities were determined according to **Jentschet al., (1996)**; **Aebi, (1974)** and **Hu (1994)** respectively.

Preparation instant coffee:

Four formula were prepared to make instant coffee by added 4gm instant coffee powder to 100 ml boiled water + 5gm table sugar, aspartame, DSAE or DDSAE respectively.

Sensory evaluation of instant coffee.

Samples of instant coffee were subjected to organoleptic tests (by fifteen judges) according to **watts et al (1989)**. Judging scale for appearance, taste, flavor, texture, crispness, colour and over all acceptability was as follow, Excellent (9-10), Very good (8 -7), Good (5-6), Fair (3-4), Poor (1-2) and very poor (0-1).

Statistical analysis

Results were expressed as the mean \pm SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (**Armitage and Berry, 1987**).

Result and discussion

Data in Table (1) showed total phenols and total flavonoids of dried stevia extracts.

Results showed that dried stevia aqueous extract (DSAE) contains high amount of total phenols and total flavonoids than that in dried debittered stevia extract by β - galactosidase enzyme (DDSAE). These results may be due to remove the bitterness of stavia may be reduce bioactive compounds in it. The result of this study were in accordance with that reported by **Thomas and Glade, (2010)** and **Benzie and Watchel-Galor, (2011)**, who reported that stevia leaf extract shows a high level of antioxidant activity, as well as the variety of phytochemicals such as phenolic compounds. Also **Edeoga et al., (2005)** reported that the most important of these bioactive constituents of stevia are alkaloids, tannins, polyphenols and flavonoids.

Sensory evaluation of stevia aqueous extract debittered by different concentrations of β -galactosidase enzyme illustrated in Table (2). There was significant ($P \leq 0.05$) decrease in bitterness, sour taste, metal taste in 5% β -galactosidase concentration compared with crude extract and the other different β -galactosidase enzyme concentrations. On the other side, there were significant increase at ($P \leq 0.05$) in sweetness and overall acceptability in 5% β -galactosidase concentration compared with crude extract by ratio of 35.77 and 25.95%, respectively. Also, there were significant ($P \leq 0.05$)

increase in sweetness and overall acceptability in 5% β -galactosidase concentration compared with the other different concentrations of β -galactosidase enzyme. Crude extract and the other different concentrations of β -galactosidase enzyme 1, 2, 3 and 4% did not significantly ($P > 0.05$) differ in their effect on sweetness. These results were in agreement with **Varuzhanet et al., (2006)** who found that stevioside possesses residual bitterness and aftertaste, which affect its qualitative characteristics. It can be eliminated by the reaction intermolecular transglycosylation of various enzymes, upon which the attachment of new carbohydrates at position C13 and C19 take place. It is the number of carbohydrate units in the above mentioned positions that determines the quality and degree of components sweetness. Finally, it could be concluded that the best concentrations of β -galactosidase enzyme was 5%. Beta-galactosidase (**Kitahateet et al., 1989**), pullulanase, isomaltase (**Ibovet et al., 1991**), and dextransaccharase (**Yamamoto et al., 1994**) are used as transglycosylating enzymes, with pullulan, maltose, lactose, and partially hydrolyzed starch, respectively, being as donors.

Table (3) showed sensory evaluation of instant coffee added with table sugar, aspartame, DSAE and DDSAE. There were no significant differences ($p > 0.05$) in all parameter between instant coffee add with table sugar and aspartame. Also there was no significant difference ($p > 0.05$) in color among all treatments. The data indicated that the worst taste, aroma, sweetness and general acceptability were observed in instant coffee add with DSAE. This is may be due to its high contents of bitterness. Treated DDSA with β -galactosidase enzyme resulting in decrease the bitterness which led to improve sensory evaluation of instant coffee.

Data presented in Table (4) illustrated the effect of DSAE, DDSAE and aspartameserum glucose of normal and hepatotoxic diabetic rats. The data showed that there was high significantly ($p \leq 0.5$) increase in the level of blood glucose for hepatotoxic diabetic rats which received basal diet alone (positive control) during duration of the experiment. Alloxan is a specific toxin that causes massive destruction of the pancreatic β -cells, provoking a state of primary deficiency of insulin without affecting other islet types, and thus creating a hyperglycaemic condition (**Arunaet et al., 1999**). However

after 3 weeks, no significant ($P>0.05$) difference was observed in blood glucose in aspartame group compared to zero time, but low significantly ($P\leq 0.05$) decreased were observed after experimental period. These results in dis agreement with **Prokić et al. (2014)** who indicated that serum concentrations of glucose increased during aspartame treatment . Furthermore, the European Prospective Investigation into Cancer and Nutrition (EPIC) has also indicated that risk for type 2 diabetes mellitus (T2DM) was elevated in those consuming at least one artificially sweetened beverages aspartame per day (**Romaguera, 2013**). On the other hand, hepatotoxic diabetic rats treated with DSAE and DDSAE were significant ($P\leq 0.05$) decreased in the level of blood glucose at 3 weeks by ratio of 8.11 and 5.66 respectively and continuously decreased to the end of experiment by ratio of 28.95 and 20.19 respectively. These results were similar to the results obtained by **Gregersen et al., (2004)** ; **Chen et al., (2005)** ; **Megejiet al. (2005)**; **Barriocanalet al., (2008)** and **Anton et al., (2010)** who reported that stevia may be advantageous in the treatment of type 2 diabetes . 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. Theses compounds are the major compounds of stevia and they exhibit anti hyperglycemic action by reducing glucose production while increasing glucose uptake to maintain plasma glucose balance (**Varanuj and Chatchai, 2009**).

Effect of DSAE, DDSAE and aspartame on liver functions of normal and hepatotoxic diabetic rats summarized in Table (5). Hepatotoxic-diabetic rats which received aspartame showed significant an elevation ($P\leq 0.05$) in AST and ALT than received the basal diet (positive control group) and other groups, while aspartame group and positive control group were similar in GGT, ALP, TP, ALb and T.bill, and there significantly increase ($P\leq 0.05$) in GGT, ALP and T. bill and decreased ($P\leq 0.05$) in ALb and TP and ALb than negative control group and stevia groups. Serum AST, ALT are the most sensitive biomarkers used in the diagnosis of liver diseases (**Junnila et al., 2000** and **Kim et al., 2000**). Recent result correlates with results reported by **Shaheen and Afifi (2014)** who found that

administration of aspartame at a dose of 500 mg/kg b.wt to rats for 42 days significantly elevated the levels of serum ALT, AST and GGT which indicated injury to the liver functions. An increase in the level of plasma total protein, decrease in albumin and increase in total bilirubin observed in aspartame treated rats could be due to increased free radical production by methanol metabolite of aspartame (**Choudhary and Devi, 2014**). On the other hand, stevia groups were significantly ($P \leq 0.05$) decreased in AST, ALT, GGT, ALP and T. Bill and increased ($P \leq 0.05$) in Alb and T.P. than other hepatotoxic diabetic groups, while DSAE group were more effective to improve liver function. In similar studies **Abdelsattaret al., (2006)** reported that stevia leaves powder and its polyphenol extract caused a significant decrease in the level of ALT and AST in diabetic treated rats.

Data presented in Table (6) summarized the effect of DSAE, DDSAE and aspartame on antioxidant status of normal and hepatotoxic diabetic rats. There were a significantly ($p \leq 0.05$) decreased in the level of GST and CAT in positive control group compared to negative group and other hepatotoxic diabetic groups. While, MDA had an opposite trend. Administration rats of CCl_4 caused to elevate level of MDA. This increase in MDA lead to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (**Patrick-Iwuanyanwu et al., 2007**). Group which taken aspartame were significantly increase in GST and decrease in MDA than positive control group while there were similar in CAT levels. Unlike the previous study **Matés (2000)** showed that during the aspartame treatment, the activity of CAT increased. Moreover, 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 MDA 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.w to rats for 42 days significantly decreased liver glutathione (GSH) are reported by **Shaheen and Afifi (2014)**. Stevia and stevia+ β -galactosidase enzyme groups were showed a significantly increased in GST and CAT compared to positive group by ratio of 35.63 and 10.51 and 44.45 and 34.31 respectively. The highest significant ($P \leq 0.05$) increase in GST and CAT was found in stevia group.

Regarding MDA, there was significantly decreased in stevia and stevia+ β -galactosidase enzyme groups compared with positive group. These results are in agree with **Yadav et al. (2012)** who showed that stevia extract possessed significant ($p \leq 0.01$) increasing of catalase activity, and decreasing ($p \leq 0.01$) in malondialdehyde level present in liver tissues.

Conclusion:

From the above results, it could be concluded that dried stevia aqueous extract (DSAE) was more effective to reduce blood glucose and improved liver functions and antioxidant status in hepatotoxic diabetic rats.

Table (1): Total phenols and total flavonoid of dried stevia extracts

	DSAE	DDSAE
Total phenols (mg GAE.g⁻¹)	21.41 ^a ± 1.17	12.22 ^b ± 1.35
Total flavonoides (mg quercetin.g⁻¹)	26.24 ^a ± 1.16	17.21 ^b ± 0.87

Each value represents the mean ±SD of three replicates. Means in the same row with different letters are significantly different ($P \leq 0.05$)

Table (2): Sensory evaluation of stevia aqueous extract debittered by different concentrations of β -galactosidase enzyme

β-galactosidase concentrations	Sweetness 40	Bitterness 40	Sour taste 10	Metal taste 10	General acceptability 100
Stevia crude extract	27.0 ^b ± 3.89	26.66 ^b ± 5.2	3.0 ^b ± 0.63	2.83 ^b ± 1.3	65.5 ^d ± 3.39
Stevia + 1% β-G.E	28.5 ^b ± 3.9	26.33 ^b ± 2.2	3.0 ^a ± 0.63	4.166 ^{ab} ± 0.75	75.0 ^b ± 3.16
Stevia + 2% β-G.E	26.16 ^b ± 2.8	28.33 ^b ± 2.6	6.5 ^a ± 1.6	3.83 ^b ± 0.98	67.66 ^{cd} ± 6.6
Stevia + 3% β-G.E	30.66 ^b ± 2.3	34.16 ^a ± 3.8	6.0 ^a ± 1.89	3.66 ^b ± 0.82	72.16 ^{bc} ± 7.3
Stevia + 4% β-G.E	27.16 ^b ± 2.5	28.0 ^b ± 4.7	5.0 ^a ± 1.26	5.33 ^a ± 1.36	61.66 ^d ± 2.6
Stevia + 5% β-G.E	36.66 ^a ± 1.6	10.83 ^c ± 0.75	2.0 ^b ± 0.89	1.16 ^c ± 0.26	82.5 ^a ± 6.89
LSD	3.51	4.16	1.48	1.16	6.322

Each value represents the mean ±SD. Fifteen judges scores. Means in the same row with different letters are significantly different ($P \leq 0.05$). β -G.E: β -galactosidase enzyme.

Table (3): Sensory evaluation of instant coffee added with table sugar, aspartame, dried DSAE and DDSAE

Coffee parameters	IC+ table sugar	IC+ aspartame	IC+DSAE	IC+DDSAE
Taste	9.67 ^a ±0.15	9.60 ^a ±0.10	6.57 ^c ±0.15	8.33 ^b ±0.15
Aroma	9.72 ^a ±0.15	9.65 ^a ±0.10	7.88 ^c ±0.80	9.17 ^b ±0.17
Bitterness	0.97 ^c ±0.02	0.96 ^c ±0.01	5.25 ^a ±0.12	1.43 ^b ±0.11
Sweetness	9.57 ^a ±0.15	9.50 ^a ±0.09	8.45 ^b ±0.23	9.66 ^a ±0.14
Color	9.77 ^a ±0.15	9.70 ^a ±0.10	9.46 ^a ±0.10	9.63 ^a ±0.18
Overall acceptability	9.90 ^a ±0.05	9.82 ^a ±0.07	7.92 ^c ±0.18	9.22 ^b ±0.17

Each value represents the mean ±SD. Fifteen judges scores. Means in the same row with different letters are significantly different (P ≤0.05). **IC: instant coffee.**

Table (4): Effect of DSAE, DDSAE and aspartame on serum glucose of normal and hepatotoxic diabetic rats

Groups	Time (week)	Control (-)	Hepatotoxic diabetic groups			
			Control (+)	DSAE	DDSAE	Aspartame
Serum Glucose (mg/dl)	0	97.7 ^{Ab} ±1.98	178.5 ^{Ba} ±1.61	183.5 ^{Aa} ±13.90	182.3 ^{Aa} ±2.73	183.0 ^{Aa} ±5.44
	3	95.0 ^{Ad} ±3.89	185 ^{ABa} ±4.47	170 ^{Bc} ±5.59	175 ^{Bbc} ±6.75	180.5 ^{Aab} ±1.18
	6	95.0 ^{Ae} ±5.58	190 ^{Aa} ±8.94	135 ^{Cd} ±6.26	145.5 ^{Cc} ±4.71	155.0 ^{Bb} ±6.26

Each value represents the means ± SD. Small different letters (a, b, c, d) in the same row significantly different (P ≤0.05) among experimental periods. Capital different letters (A, B, C, D) in the same column significantly different (P ≤0.05) among groups.

Table (5): Effect of DSAE,DDSAE and aspartame on liver functions of normal and hepatotoxic diabetic rats.

Parameters	Control (-)	Hepatotoxic diabetic groups			
		Control (+)	DSAE	DDSAE	Aspartame
AST (U/L)	52.5 ^c ±2.05	92.0 ^b ±1.78	60.0 ^d ±5.58	72.0 ^c ±6.75	106.6 ^a ±2.71
ALT (U/L)	42.0 ^c ±4.73	66.0 ^b ±2.36	51.0 ^d ±5.86	60.0 ^c ±3.22	77.0 ^a ±3.22
GGT (U/L)	40.0 ^d ±3.22	62.0 ^a ±2.68	45.0 ^c ±2.36	52.0 ^b ±4.47	60.08 ^a ±1.81
ALP (U/L)	54.33 ^d ±1.37	80.0 ^a ±2.68	55.5 ^d ±4.40	60.4 ^c ±3.94	81.0 ^a ±2.38
Total bilirubin(mg/dl)	0.23 ^d ±0.22	1.2 ^a ±0.36	0.73 ^b ±0.03	0.78 ^b ±0.09	1.05 ^a ±0.04
albumin(mg/dl)	4.0 ^a ±0.45	1.2 ^c ±0.18	3.3 ^b ±0.28	3.2 ^b ±0.36	1.38 ^c ±0.20
TP (mg/dl)	6.0 ^a ±0.64	3.0 ^c ±0.56	5.5 ^{ab} ±0.45	5.2 ^b ±0.71	2.8 ^c ±0.45

Each value represents the means ± SD. Different letters in the same row were significantly different (P ≤ 0.05).

Table (6): Effect of DSAE,DDSAE and aspartame on antioxidant status of normal and hepatotoxic diabetic rats.

Parameter	Control (-)	Hepatotoxic diabetic groups			
		Control (+)	DSAE	DDSAE	Aspartame
GST(U/mg)	304.0 ^a ±3.2	185.5 ^c ±3.4	251.6 ^b ±3.7	227.0 ^c ±6.49	205.0 ^d ±4.5
CAT(U/mg)	110.9 ^a ±0.9	68.05 ^d ±1.9	98.3 ^b ±1.5	91.4 ^c ±2.01	69.29 ^d ±1.4
MDA(nmol/mg)	21.6 ^d ±1.5	42.36 ^a ±1.9	30.4 ^c ±2.2	32.7 ^c ±1.82	38.0 ^b ±2.4

Each value represents the means ± SD. Different letters in the same row were significantly different (P ≤ 0.05).

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تأثير مستخلصات الاستيفيا و الاستيفيا مزالة المرارة على الفئران المصابة بارتفاع سكر الدم مع التسمم الكبدى

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الملخص العربي:

الاستيفيا ، نبات محلى طبيعى له العديد من الخصائص العلاجية و التجارية على مستوى العالم ، لذلك تهدف هذه الدراسة الى تقييم تأثير مستخلصات الاستيفيا و الاستيفيا بعد ازالة مرارتها على الحد من اثار اصابة الفئران بارتفاع سكر الدم مع تسمم الكبد. تم إضافة تركيزات مختلفة ١، ٢، ٣، ٤، ٥ % من انزيم بيتا جلاكتوسيديز الى المستخلص المائى من الاستيفيا بغرض ازالة المرارة من الاستيفيا. وأظهرت النتائج انمستخلص الاستيفيا المائى المعامل ب٥% من انزيم البيتا جلاكتوسيديز قد سجل احسن النتائج الحسية. تم إعداد قهوة سريعة التحضير بإضافة سكر المائدة، المستخلص المائى المجفف من الاستيفيا و المستخلص المائى المجفف من الاستيفيا المزال مرارته و الاسبارتام و انالتقييم الحسى للقهوة لوحظ ان القهوة المضاف اليها المستخلص المائى المجفف من الاستيفيا المزال مرارته كانت احسن فى كل من الحلاوة و المرارة و القبول العام بالمقارنة بباقي الاضافات. تم استخدام اربعون فأراً بالغاً تم تقسيمهم الى مجموعتين رئيسيتين ، المجموعة الاولى (المجموعة الكنترول السالبة) عددها ٨ فئران ، المجموعة الثانية وتم اصابتها بتسمم الكبد مع مرض السكرى (ن=٣٢)، تم تغذيتهم على الوجبة الرئيسية و تقسيمهم الى اربع مجموعات فرعية (ن=٨)، المجموعة الفرعية الاولى و هى المجموعة الكنترول الموجبة و المجموعات الفرعية الثانية و الثالثة و الرابعة تم إعطائهم بالحقن عن طريق الفم جرعة يومية (٤٠٠ ملجم /كجم من وزن الجسم) من المستخلص المائى المجفف من الاستيفيا و المستخلص المائى المجفف من الاستيفيا المزال مرارته و الاسبارتام على التوالي. و فى نهاية التجربة تم ذبح الفئران و جمع عينات الدم و المصل للتحليل. أشارت النتائج الى أن الفئران التى عولجت بالمستخلص المائى المجفف للاستيفيا و المستخلص المائى المجفف للاستيفيا المزال مرارته بواسطة انزيم البيتا جلاكتوسيديز ادت الى خفض مستويات جلوكوز الدم و انزيمات الكبد الاسبارتات امينوترانسفيريز و الالانين امينوترانسفيريز و الفوسفاتيز القاعدى و البليوروبين الكلى و ادت الى تحسين الحالة التأكسدية و الوزن المكتسب و لكن المستخلص المائى المجفف للاستيفيا كان اكثر فاعلية. و خلاصة القول، كان استخدام مستخلصات الاستيفيا فى العلاج ادى الى التقليل من معدل جلوكوز و اكسدة الدهون و تحسين و وظائف الكبد فى فئران السكرى المصابة بتسمم الكبد.

الكلمات الكاشفة: الاستيفيا - الاستيفيا المزال مرارته - فئران السكرى المصابة بتسمم الكبد - انزيم البيتا جلاكتوسيديز - القهوة سريعة التحضير .