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Comparative Study on the Effects of Steviosides and Aspartame on Glucose, Urea and Creatinine Levels of Normal and Type 2 Diabetic Rats

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ABSRACT: Comparing the effects of the natural nonnutritive sweetener (steviosides) and the artificial sweetener (aspartame) on the plasma glucose, urea, and creatinine levels of normal and type 2 diabetic rats revealed that treating normal and diabetic rats with different doses of both sweeteners reduced the plasma glucose levels except in normal rats treated with low dose of aspartame and high dose of steviosides that increased glucose levels by 17.3% and in normal rats treated with the high aspartame dose (3.8%) but fortunately they were still within the normal glucose range. All doses of both sweeteners increased urea levels in normal rats by percentages ranging from 5.2% to 41.7% though they were within the normal urea range except the low aspartame dose and high steviosides dose, moreover medium aspartame dose reduced urea level by 11.1%. All doses of both sweeteners reduced the urea levels in diabetic rats with the highest reduction percentage in those treated with the high steviosides dose (63.8%) while the lowest (40.9%) was in those treated with the medium dose of aspartame but unfortunately, no dose succeeded to lower urea levels to their normal ranges. Treating normal rats with different doses of both sweeteners increased the plasma creatinine by percentages ranging from 33.3% in the medium steviosides dose to 133.3% in the low aspartame dose although they were kept within the normal creatinine range. Treating diabetic rats with different doses of both sweeteners succeeded to lower creatinine levels to their normal ranges with reduction percentages ranging from 25.8% to 38.1%. The creatinine levels were more or less similar in diabetic rats treated with different doses of both sweeteners with no significant differences between the two sweeteners in any dose.

KEY WORDS: Nonnutritive Sweeteners, Aspartame, Steviosides, Plasma Glucose, Urea, Creatinine.

INTRODUCTION

Avoiding concentrated sources of sugar is important for healthy and diabetic persons in improving health and maintaining appropriate blood sugar level. One way to control excessive energy intake is to replace the calories from sugar with a nonnutritive

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sweetener, allowing the pleasure of a sweet taste without consuming additional calories. Scientists have responded to the increased consumer demand of sweeteners by developing, researching, and producing a number of energy-reduced or nonnutritive sweeteners.[1] Nonnutritive sweeteners can offer consumers a way to enjoy the taste of sweetness with little or no energy and/or glycemic response. Nonnutritive sweeteners can assist in weight management, control of blood glucose, and prevention of dental caries.[2] Aspartame is one of the most widely used artificial sweeteners. It is 160–220 times sweeter than sucrose and gives 4 kcal/g but the quantity used is so low that it is regarded as calories free.[3] The safety of aspartame has been repeatedly questioned and associations between its intake and some ailments such as headache, nausea, depression, tiring, irritability, tachycardia, insomnia, dizziness, memory loss, ..,etc. have been hypothesized.[4-7] It was even supposed that aspartame could be a risk factor for brain cancer and could worsen some pathologies as multiple sclerosis, epilepsy, etc…,[8-10] *Stevia* is the generic term used for food ingredients derived from the herb *Stevia rebaudiana* (Bertoni). In few countries, *Stevia* has been consumed as a food and medicine for many years, including most notably Japan and Paraguay.[11] *Stevia* leaf or extracted form has been used in the traditional medicine for the treatment of diabetes but still not approved by the FDA as a sweetener.[12] In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most

patients will likely be found by 2030.[13] Accordingly, this study was undertaken to compare the effects of consumption of steviosides extracted from *Stevia rebaudiana* (Bertoni) leaves (as a natural sweetener) and aspartame (as an artificial sweetener) on plasma glucose, urea and creatinine levels of normal and type 2 diabetic rats.

MATERIAL AND METHODS

A total of 140 male Wistar rats were divided into two equal categories. The first category (normal category) consisted of seven groups (each of 10 rats). Rats of the second category were injected subcutaneously with alloxan (120 mg/kg BW) for the induction of type2 diabetes.[14] After stabilization of type 2 diabetes for one week, rats were considered diabetic and divided randomly into seven groups (each of 10 rats) representing the diabetic category. The first group of the first category was considered as a control group while the first group of the diabetic category was considered as untreated diabetic group. The other six groups of each category were treated with steviosides and aspartame with three different doses. The Low steviosides dose was 7.9 mg/kg/day and representing its ADI whereas its medium and high doses were 16 and 32 mg/kg/day and representing double and four times its ADI. In case of aspartame, the low dose was 50 mg/kg/day and representing its ADI of the FDA, while its medium and high doses were 100 and 200 mg/kg/day and representing double and four times its ADI. Rat groups were orally administered their respective doses of each sweetener in their drinking water where the daily water consumption and doses of each sweetener designated to each group were calculated. At the end of 4 weeks experimental period, rats were fasted for 12 hours and blood specimens were collected from each rat into a separate heparin tube and centrifuged. The collected plasma samples

were subjected to the followings tests using kits

from Diamond Diagnostic Company:

1. **Determination of plasma glucose levels** according to Hyvarinen and Nikkila.[15]
2. **Determination of plasma urea levels** according to Patton and Crouch.[16]
3. **Determination of plasma creatinine levels** according to Henry *et al.*[17]

RESULTS

Table (1) shows that treating normal and diabetic rats with different doses of each of aspartame and steviosides reduced the plasma glucose levels except in normal rats treated low dose of aspartame and high dose of steviosides that increased glucose levels by 17.3% and in normal rats treated with the high aspartame dose (3.8%) but fortunately they were still within the normal range (55-135 mg/dl). There were no significant differences in plasma glucose levels of normal rats between both sweeteners in any dose. Treatment of diabetic rats with different doses of steviosides resulted in lower plasma glucose levels than the corresponding aspartame doses that were significant in case of low and medium doses. The highest decrease percentage in diabetic rats (62.6%) was in those treated with high steviosides dose while the lowest was in those treated with the medium aspartame dose (37.8%).

Table (2) illustrates that all doses of both sweeteners increased urea levels in normal rats by percentages ranging from 5.2% to 41.7% though they were within the normal range (15 - 45 mg/dl) except the low aspartame dose (51.8 mg/dl) and high steviosides dose (49.2 mg/dl). Moreover medium aspartame dose reduced urea level by 11.1%. Treating normal rats with the low doses of steviosides resulted in lower urea levels than the corresponding aspartame doses, although its other two doses induced higher levels. Moreover, the

difference in urea levels between the two sweeteners was significant only in case of the medium dose. All doses of both sweeteners reduced the urea levels in diabetic rats with the highest reduction percentage in those treated with the high steviosides dose (63.8%) while the lowest (40.9%) was in those treated with the medium dose of aspartame but unfortunately, no dose succeeded to lower urea levels to their normal ranges. Treating diabetic rats with the medium and high steviosides doses resulted in lower urea levels than the corresponding aspartame doses and the differences between the two sweeteners were statistically insignificant in all doses.

Table (3) demonstrates that treating normal rats with different doses of both sweeteners increased the plasma creatinine by percentages ranging from 33.3% in the medium steviosides dose to 133.3% in the low aspartame dose although they were kept within the normal creatinine range (0.2- 0.8 mg/dl). Different doses of steviosides resulted in lower creatinine levels than the corresponding aspartame doses although the difference was significant only in the low dose. Moreover, creatinine levels remained unchanged in the low steviosides dose. Treating diabetic rats with different doses of both sweeteners succeeded to lower creatinine levels to their normal ranges with reduction percentages ranging from 25.8% to 38.1%. The creatinine levels were more or less similar in diabetic rats treated with different doses of both sweeteners with no significant differences between the two sweeteners in any dose.

DISCUSSION

High intensity sweeteners can offer consumers a way to enjoy the taste of sweeteners with little or no energy and/or glycemic response.[18] The doubts about the efficacy and safety of oral hypoglycemic agents have prompted a search for safer and more effective natural products for

treatment of diabetes.[19] To the best of our knowledge, this is one of the leading studies that compares between the effects of steviosides and aspartame from the biochemical point of view using different doses of each sweetener.

1. Impact of steviosides and aspartame on plasma glucose levels

In diabetics, nonnutritive sweeteners either natural or artificial may help to keep blood sugar levels under control. Extracts of leaves from the plant *Stevia* have been used for many years in traditional medicine in South America in the treatment of diabetes.[20] The present study showed that although treating diabetic rats with different doses of steviosides lowered the plasma glucose levels than that of untreated diabetics by percentages ranging from 55.6% to 62.6%, they were still above the normal plasma glucose range. It is worth mentioning that the decline in the glucose levels were dose dependent (table 1). A previous study showed that oral intake of extracts from *Stevia* plant for 3 days resulted in 35% reduction in blood glucose levels in diabetic subjects. Another study found an increase in fasting blood glucose in diabetic patients receiving placebo but not in those receiving 500 mg or one gram steviosides 3 times daily for 3 months. This lowering effect of the steviosides may be attributed to its ability to regulate plasma glucose levels by enhancing not only insulin secretion but also insulin utilization in diabetic rats and/or its action directly on pancreatic beta cells to secrete insulin.[21] Other study concluded that steviosides exerts antihyperglycemic via insulinotropic and glucagonostatic actions in the type 2 diabetic rats, and may have the potential of becoming a new antidiabetic drug for use in type 2-diabetes.[22] Our study revealed that the plasma glucose levels of normal rats were fluctuating from the control level although they were kept within the normal range (55-135 mg/dl) and this is supported by another

study that revealed that there was a relatively low risk of hypoglycemia in normal subjects from consumption of dietary concentrations of steviosides so it was suggested that the reductions only occur when blood glucose concentrations are elevated, as in the diabetic state.[23] Another study found no effect on blood glucose in healthy human subjects after steviosides treatment with 250 mg 3 times daily for 1 year.[24]

Treating diabetic rats with different doses of aspartame significantly reduced the mean plasma glucose level. The percentage reduction reaches more than half (59.7%) of the untreated diabetic level in its high dose (table 1). This is not consistent with another study reporting that aspartame kept blood glucose level out of control and may cause patients to go through coma.[25] The effect of aspartame on plasma glucose level could be attributed to its potent sweetening power that stimulates taste receptors which in turn enhanced insulin production, thus reduced the blood glucose level. Treating normal rats with aspartame kept plasma glucose level within its normal range (table 1). This is consistent with a study examined the blood sugar response to aspartame-sweetened beverages in human and found no effects of different doses.[26] Our study revealed that different doses of steviosides were significantly more efficient in lowering plasma glucose levels in diabetic rats than the corresponding doses of aspartame.

2. Impact of steviosides and aspartame on kidney function

The deterioration that characterizes kidney disease of diabetes takes place in and around the glomeruli. Early in the disease, the filtering efficiency diminishes, and important blood proteins are lost in the urine. later, the kidneys lose their ability to remove waste products, such as creatinine and urea that when measured in the blood gives an indication of how far a kidney disease has progressed.[27] The present

study revealed that treating normal rats with both sweeteners increased urea and creatinine levels but fortunately they were within the normal ranges in most tried doses. The highest increase in urea (41.9%) and creatinine level (133.3%) were in those treated with the low dose of aspartame (tables 2 and 3). Another study revealed that subcutaneous injection of rats with steviosides (1.5 g/kg BW) increased the blood urea nitrogen (BUN) and creatinine levels by 180% and 132%, respectively after 9 hours and histopathological examination of the kidney revealed degeneration of the proximal convoluted tubule cells.[28] It is worth mentioning that the dose used in the study was about 50 times much higher than our high oral steviosides dose.

The present study showed that treating diabetic rats with different doses of steviosides and aspartame improved the kidney function and succeeded to reduce the creatinine levels to their normal range (tables 2 and 3). It can be concluded that the medium and high steviosides doses were insignificantly more efficient in improving urea level in diabetic rats than the corresponding aspartame doses while its medium dose was insignificantly more efficient in improving creatinine levels.

RECOMMENDATIONS

1. Steviosides can be used safely for diabetic patients due to its antihyperglycemic effect.
2. Further laboratory animal studies on the effects of combinations of different doses of steviosides and aspartame are required.
3. Further human and laboratory animal studies on the effects of different doses of steviosides and aspartame on diabetics beside the conventional treatment are recommended.

Table (1): Plasma glucose levels (mean ±SD) in normal and diabetic rats treated with different doses of steviosides and aspartame.

Dose	Sweetener	Normal group			Diabetic group		
		Glucose level (mg/dl)	% [a]	T-test[b]	Glucose level (mg/dl)	% [a]	T-test[b]
Low	Steviosides	101.4 ± 7.9	-2.7 %	4.671	196.4 ± 32.1	-55.6%	3.075*
	Aspartame	122.6 ± 4.3	17.3%		253.7 ± 31.7	-42.8%	
Medium	Steviosides	102.4 ±23.4	-1.7 %	1.341	180.4 ± 9.5	-59.3%	2.768*
	Aspartame	88.0 ± 5.2	-3.9 %		275.6 ± 66.4	-37.8%	
High	Steviosides	122.6 ±29.8	17.3%	0.841	165.3 ± 40.5	-62.6%	0.033
	Aspartame	108.4 ±23.1	3.8 %		178.0 ± 55.3	-59.7%	
Control & (Untreated diabetic)	-	104.2±14.6	-	-	(442.0±31.1)	-	-

[b] Compares the mean test levels between steviosides and aspartame in each dose.
* P<0.05.
[a] % of increase or decrease from the control or diabetic untreated level.

Table (2): Plasma urea levels (mean ±SD) in normal and diabetic rats treated with different doses of steviosides and aspartame.

Dose	Sweetener	Normal group			Diabetic group		
		Urea level (mg/dl)	% [a]	T-test[b]	Urea level (mg/dl)	% [a]	T-test[b]
Low	Steviosides	38.4 ± 2.6	5.2%	1.477	72.0 ± 18.6	-43.3%	1.794
	Aspartame	51.8 ± 13.1	41.7%		53.1 ± 15.9	-58.2%	
Medium	Steviosides	43.6 ± 9.1	19.4%	2.308*	54.5 ± 12.6	-57.0%	1.115
	Aspartame	32.8 ± 5.2	-11.1%		75.2 ± 14.6	-40.9%	
High	Steviosides	49.2 ± 13.4	36.1%	1.155	46.2 ± 12.0	-63.8%	1.551
	Aspartame	38.6 ± 8.8	5.6%		51.7 ± 5.2	-59.0%	
Control & (Untreated diabetic)	-	36.5 ± 2.6	-	-	(127.8±28.7)	-	-

[b] Compares the mean test levels between steviosides and aspartame in each dose.
* P<0.05.
[a] % of increase or decrease from the control or diabetic untreated level.

Table (3): Plasma creatinine levels (mean ±SD) in normal and diabetic rats treated with different doses of steviosides and aspartame.

Dose	Sweetener	Normal group			Diabetic group		
		Creatinine level (mg/dl)	% [a]	T- test[b]	Creatinine level (mg/dl)	% [a]	T-test[b]
Low	Steviosides	0.3 ± 0.1	0.0%	3.329*	0.7 ± 0.1	-25.8%	0.980
	Aspartame	0.7 ± 0.3	133.3%		0.6 ± 0.2	-38.1%	
Medium	Steviosides	0.4 ± 0.1	33.3%	1.932	0.6 ± 0.2	-38.1%	0.920
	Aspartame	0.6 ± 0.1	100.0%		0.7 ± 0.1	-25.8%	
High	Steviosides	0.5 ± 0.0	66.7%	1.805	0.7 ± 0.1	-25.8%	0.769
	Aspartame	0.6 ± 0.1	100.0%		0.7 ± 0.1	-25.8%	
Control & (Untreated diabetic	-	0.3 ± 0.1	-	-	(0.9 ± 0.2)	-	-

[b] Compares the mean test levels between steviosides and aspartame in each dose.
* P<0.05.
[a] % of increase or decrease from the control or diabetic untreated level.

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