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Full Length Research Paper

Biological properties of *Stevia* sweetener and egg replacers' products on serum biochemical markers of diabetic rats

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The aim of this study was to evaluate the biological effect of formulated diet containing wheat gluten and *Stevia* as egg and sweetener replacers in chemically induced diabetic rats. Diabetic rats were fed on either basal diet (BD) or formulated diet (FD) containing wheat gluten and *Stevia* for 30 days. Fasting plasma were subjected to analysis of glucose level biochemical markers as lipid profile, liver and kidney function tests. The results obtained showed that the FD for diabetics has a good score. Compared with the BD, the high-protein diet resulted in lower serum concentrations of triacylglycerol ($p < 0.05$), glucose ($p < 0.001$) and higher serum concentrations of urea ($p < 0.001$). No significant differences were detected in total or HDL cholesterol or in creatinine. There was a slight decrease in urea and AST values in FD for diabetics group compared with BD. Formulated diet containing gluten protein with *Stevia* reversed the effects of diabetes on reducing glucose, LDL-c, triacylglycerol levels and can be considered as a valuable candidate in the reversal of the diabetic complications.

Key words: *Stevia*, wheat gluten, diabetes, rats.

INTRODUCTION

Stevia is stable, non-caloric, maintains good dental health by reducing the intake of sugar and opens the possibility for use by diabetic and phenylketonuria patients and obese persons. Gluten is an insoluble protein found in wheat and other grains. Consumption of high quantities of fat and sugar are the major nutritional problems facing mankind in this century which has been associated with serious health problems, especially diabetes (Jeppesen et al., 2002). Many studies have been performed to produce low calorie products using wheat flour, wheat bran and/ or maltodextrin and fructose (Leibowitz and Cerasi, 1996). *Stevia rebaudiana* (Bertoni) (*Asteraceae*) is a sweet herb. Stevioside, which is the major sweet substance of this plant (5 - 10% of dry weight) is 300 times as sweet as sucrose. It has steviol as its aglycone and is attached to three glucose molecules (Chan et al., 2000). The leaves of *S. rebaudiana* also contain several structurally related compounds such as rebaudioside A - E, dulcoside A and steviolbioside, several of which are sweet. *Stevia* sweetener, the crude extract from leaves, has been used for a few decades to sweeten beverages (Krarup et al., 1983).

The dry extract from *Stevia* leaves also contains flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycinnamic acids (Caffeic, chlorogenic, etc.), neutral water soluble oligosaccharides, free sugars, amino acids, lipids, essential oils and trace elements (Kinghorn et al., 2001). *Stevia* sweetener extracts have been suggested to exert beneficial effects on human health, including antihypertensive (Carakostas et al., 2008), antihyperglycemic and anti-human rotavirus activities (Komissarenko et al., 1994). The advantages of stevioside as a dietary supplement for human subjects are manifold: it is stable, non-caloric, maintains good dental health by reducing the intake of sugar and opens the possibility for use by diabetic and phenylketonuria patients and obese persons (Chan et al., 2000).

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Gluten is an insoluble protein found in wheat and other grains (Payne et al., 1987). Wheat gluten, the bioproduct of the wheat starch industry, is a typical water insoluble protein consisting of more than 60 different molecular mass polymorphic polypeptides. Of the gluten proteins, gliadin has a relative molecular mass between 30,000 and 80,000 Dalton, whereas glutenin is multi-chained with relative molecular mass up to several million Dalton (Shewry et al., 1992). The low solubility of wheat gluten limits its utilization in food industry. The improvement of the functional properties of the proteins by enzymatic or chemical modification has been widely conducted (Bites and Lookhart, 1996). Linares et al. (2000) reported that mild acid treatment caused the increase in solubility of wheat gluten. Kato et al. (1989) found that deamidation of gluten by proteolytic enzymes was effective in increasing its functional properties. Limited enzymatic hydrolysis using proteases resulted in improved solubility and properties of emulsion and foam of wheat gluten. Subjected to limited hydrolysis by chymotrypsin, the obtained peptides could be separated. The emulsifying and foaming properties of gluten treated with protease (chymotrypsin, papain, pronase and pepsin) or using mild acid, followed by microbial transglutaminase cross-linking, were improved greatly (Lens et al., 1999).

Diabetic subjects suffered from impaired metabolism of carbohydrate, fats and proteins. In addition, diabetic affects different body functions. Therefore, this study was undertaken to investigate the biological effects of formulated diet containing wheat gluten and *Stevia* powder compared with basal diet as a great replacement diet for diabetics' rats.

MATERIALS AND METHODS

Animals

The experimental animals were divided into four groups (ten rats each) as follow:

Group I (control +BD)

The animals were injected intraperitoneally (i.p.) for five consecutive days with 100 ul saline daily and fed on basal diet (BD).

Group II (control +FD)

The animals were injected i.p. for five consecutive days with 100 ul saline daily and fed on formulated diet (FD). The animals (in groups III and IV) were injected daily with alloxan (75 mg/kg/ day) i.p. for five consecutive days. Blood sugar was measured to confirm the development of diabetes mellitus (DM) (mean ± SD of fasting blood sugar baseline was 295 ± 15 mg /dl).

Group III (diabetic + basal diet)

The diabetic animals were fed on basal diet (BD).

Group IV (diabetic + FD)

The diabetic animals were fed on formulated diet (FD) and the dose of alloxan for induction of diabetes was given according to Suresh and Das (2003).

The composition of BD

Casein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%) and starch (64%).

The composition of FD

FD was composed of wheat gluten (12%), *Stevia* (1%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%) and starch (68%). *Stevia* was obtained from local market, while wheat gluten was obtained from Bioline Company at Jeddah. The feeding experiment was continued for 30 days and at the end of the experimental period, animals were weighted and anesthetized by exposure to an atmosphere of 100% diethyl ether and killed by decapitation. Blood samples were taken into plain tubes. A blood sample was collected on plain tubes for separation of serum after centrifugation at 3000 rpm for 15 min. Serum glucose level was determined after sampling. The remainders were kept at -20°C.

Methods

Serum glucose, serum total cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triacylglycerol (TG), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) activities, urea and creatinine were determined according to the methods described in commercially available kits obtained from Bioline diagnostic kits.

Statistical analysis

Mean, standard deviation and coefficient of variation of the obtained data from each different experimental group were calculated using SPSS program version 8.0 and $p < 0.05$ was considered significant.

RESULTS

Table 1 showed that serum glucose level of the diabetic rats which consumed basal diet (BD) was significantly greater than that of rats which consumed formulated diet (FD) ($p < 0.001\%$). Serum glucose level of control rats which consumed BD was not significantly greater than that which consumed FD. It was observed that, non significant changes in the level of serum total protein and the activity of AST in control or diabetic rats either fed on BD or FD (Table 1). Compared with the BD, the high-protein diet resulted in lower serum concentrations of triacylglycerol ($p < 0.05$), glucose ($p < 0.001$) and higher serum concentrations of urea ($p < 0.001$). Significant differences were not detected in total or HDL cholesterol or in creatinine. There was a slight decrease in urea and AST values in FD for diabetics group when compared with BD.

In this study, serum ALT, urea and creatinine were

Table 1. Serum levels of glucose, total protein, ALT, AST, urea and creatinine in the studied groups (Mean ±SD).

Groups / Marker serum	I	II	III	IV
Glucose (mg/dl)				
Mean±SD	78 ± 4.5	67 ± 3.3	201 ± 10	112 ± 5.1
P value	---	<0.001	<0.01	<0.001
P*		---	<0.001	<0.001
Total protein (mg/dl)				
Mean±SD	5.9 ± 0.9	5.5 ± 0.6	5.1 ± 0.5	6.1 ± 0.9
P value		N.S.	N.S	N.S.
P*	---	--	N.S	N.S.
AST(U/L)				
Mean±SD	25 ± 2.3	24 ± 2.0	26 ± 2.5	23 ± 2.6
P value		N.S	N.S	N.S
P*			N.S	N.S
ALT(U/L)				
Mean±SD	22 ± 2.1	20.7 ± 1.1	33 ± 2.2	23 ± 2.5
P value	---	<0.05	<0.05	N.S
P*		--	<0.05	N.S.
Urea (mg/dl)				
Mean±SD	31 ± 3.2	28 ± 1.8	39 ± 2.0	27 ± 3.1
P value		N.S.	<0.05	N.S.
P*		--	<0.01	N.S.
Creatinine (mg/dl)				
Mean±SD	0.8 ± 0.16	0.9 ± 0.03	1.3 ± 0.34	0.91 ± 0.04
P value	---	<0.001	<0.05	N.S
P*		--	<0.01	N.S

I: Control + BD. II: Control + FD. III: Diabetic + BD. IV: Diabetic + FD. P: compared with control group. P*: formulated diet versus basal diet. P<0.05 was considered as significant. N.S.: non significant

significantly higher in diabetic rats fed on BD when compared with control group ($p < 0.001$% each). On the other hand, diabetic rats fed on FD showed a significant reduction of these parameters when compared with BD. Lipid profile of normal and diabetic rats fed on either BD or FD was shown in Table 2.

Results obtained showed that, serum total cholesterol was significantly higher in control group fed on BD compared with FD ($p < 0.01$), while there were no significant changes in the levels of triglycerides, HDL-c, LDL-c and VLDL-c.

Diabetic rats fed on BD showed a significant elevation in serum total cholesterol and LDL-c, while HDL-c was significantly decreased as compared with diabetic rats fed on FD (Table 2).

DISCUSSION

At present, there is a sharp increase in the incidence of type 2 diabetes mellitus and obesity as a result of aging, dietary habits and decreasing physical activities. These metabolic syndromes have become major public health problems in industrialized and developing countries. Type 2 diabetes mellitus is a chronic metabolic disorder resulting from defects in both insulin secretion from β-cells of islets and insulin action (Jeppesen et al., 2000). Currently, there is a popular use of herbal and alternative medicine for the treatment of diabetes. Indeed, extract from *S. rebaudiana* has long been used for the treatment of diabetes in South America (Das et al., 1992). In addition, stevioside, the major component of the extract,

Table 2. Serum levels of total-cholesterol, triacylglycerol, HDL-C, LDL- C and VLDL-C in the studied groups (Mean ±SD).

Groups / Serum marker	I	II	III	IV
T-cholesterol (mg/dl)				
Mean±SD	62 ± 3.1	59 ± 0.21	79 ± 0.5	68 ± 0.19
P value	---	<0.001	<0.05	N.S
P*		---	<0.01	<0.01
Triacyglycerol (mg/dl)				
Mean±SD	78 ± 3.9	72 ± 1.4	90 ± 4.5	77 ± 2.2
P value	---	N.S	<0.05	N.S.
P*			<0.001	<0.05.
HDL-c (mg/dl)				
Mean±SD	28 ± 1.3	30 ± 2.7	22 ± 2.1	27 ± 3.3
P value	---	N.S	<0.05	N.S
P*		--	<0.05	<0.05
LDL-c (mg/dl)				
Mean±SD	17 ± 1.1	15 ± 1.9	30 ± 4.3	20 ± 2.2
P value		N.S	<0.001	N.S
P*		---	<0.001	<0.001
VLDL-c (mg/dl)				
Mean±SD	12 ± 1.0	10 ± 1.5	15 ± 1.2	16 ± 1.5
P value		<0.05	<0.05	N.S.
P*		--	<0.01	<0.05

I: Control + BD. II: Control + FD. III: Diabetic + BD. IV: Diabetic + FD. P: compared with control. P*: formulated diet versus basal diet. P<0.05 was considered as significant. N.S.: non significant.

has a high sweetness with no calorie and only a small amount is needed for sweetening purposes. Thus, it should be a good alternative to sugar for diabetic patients. An early study showed that 0.5 g % of stevioside and 10 g % of powdered *Stevia* leaves in both high-carbohydrate and high-fat diets given to rats caused a significant reduction in blood glucose level following 4 weeks of treatment (Takahashi et al., 2001). Subsequently, the effect of aqueous extract of *Stevia* leaves on glucose tolerance test was investigated in humans. Following the intake of aqueous extract of *Stevia* leaves (5 g % at 6 h intervals for 3 days), there was a significant decrease in plasma glucose level during glucose tolerance test and after overnight fasting in all healthy subjects (Geuns, 2003). These observations support the earlier notion that stevioside and *Stevia* extract can be used to treat diabetic condition.

The use of *Stevia* as a replacement of sucrose and the wheat gluten as a source of protein in the diet of diabetic rats for 30 days has allowed the determination of the relative effects on serum glucose, lipid profile, liver and kidney functions of diabetic rats. *Stevia* has been reported to reduce serum glucose level in humans and animals (Chan et al., 2000); however, other authors have reported that it was ineffective in lowering serum cholesterol in patients with hypercholesterolemia (Melis, 1992). *Stevia* suppresses the fasting blood glucose level in diabetic rats by an average of 40% compared with basal diet. Previous studies in rats have indicated that stevioside may increase urinary glucose loss as well as augmenting liver glycogen storage (Usami et al., 1980). The reason for the apparent discrepancy is not clear. The

present study is unable to clarify if stevioside has direct effects on the peripheral glucose disposal beside that induced by insulin. There are no indications from this study that *Stevia* would cause late hypoglycemia. In the present study, *Stevia* was administered orally as 91% pure *Stevia*. Therefore, the authors cannot totally exclude the possibility that other substances in the extract, in part, could be responsible for the observed effects. Triglyceride level was not changed by *Stevia* indicating that stevioside does not affect the intestinal nutrient uptake. Corroborating this, *Stevia* has been found not to alter the uptake of oleic acid from the rat intestine (Aze et al. 1991).

There is evidence that stevioside may have beneficial actions on other constituents of the metabolic syndrome than the hyperglycemia. Stevioside seems to be a safe compound that has been used for decades as a sweetening agent in several countries such as Japan without adverse effects. Whether long-term administration of stevioside would improve the postprandial glycemia and stevioside or not is considered to be a sugar substitute and commercial sweetener, both in the form of stevioside and *Stevia* extract (Wang et al., 1998).

They are used in a variety of foods and products, such as pickled vegetables, dried seafood, soy sauce, beverages, candies, chewing gum, yogurt and ice cream, as well as in toothpaste and mouth wash. Results of the present study conclusively demonstrated that consumption of formulated diet (FD) was effective in lowering serum glucose and total cholesterol. Numerous studies have shown that, *Stevia* was effective in lowering the total cholesterol levels in rats and humans (Melis, 1992). It has been suggested that soluble dietary fibers decrease serum cholesterol by binding or entrapping bile acid resulting in a decreased absorption and increased excretion of the bile acid in feces. One explanation for the decrease in serum cholesterol, associated with consumption of FD, relates to the decreased food consumption. The results of the present study clearly show that consumption of FD, which reduced energy intake of the rats fed soluble dietary fiber, also had proportionately less of all dietary nutrients. Likewise, the overall reduction in the energy consumed, correlated significantly with the lower serum concentration of triglycerides and glucose. Liver and kidney function of control and diabetics rats fed on either BD or FD are shown in Table 2. A significant decrease in ALT, urea and creatinine values was found in diabetics rats fed on FD compared to that fed on BD. There was a slight increase in HDL-c in diabetic rats fed on FD compared with those fed on BD. This study is in agreement with Linares et al., 2000. They found that low doses had no effect on total cholesterol, triglycerides, AST, ALT, protein and glucose in serum. However, at high doses, results showed a slight increase in the above tested parameters when compared to the low doses. It was concluded that *Stevia* sugar can replace sucrose in diet and wheat gluten can replace protein to produce and formulate a functional meal for diabetics with low calories and as a result, control diabetics. Following up, liver and kidney function in screening any side effects.

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