ANTIHYPERGLYCEMIC EFFECTS OF STEVIOSIDE ON DIABETIC RATS

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ABSTRACT

Stevioside as a natural sweetener was extracted from leaves of plant \textit{Stevia rebaudiana Bertoni}, and evaluated in alloxan-induced diabetes in rats. Results showed that oral administration of 500 mg kg\textsuperscript{-1} body weight of the stevioside for successive three weeks produced, significant reduction in blood glucose in diabetic rats. These results were compared with glibenclamide (600 \(\mu\)g/kg, orally) as a standard antihyperglycaemic agent. Also, a deformation of pancreatic b-cells was observed in the diabetic rats treated with stevioside. This indicates that stevioside may be a promising antidiabetic agent in treatment of type 2 diabetes. Stevioside was proved to be a potential drug or food additive for improving diabetes regulation.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by abnormally elevated levels of glucose in blood and urine. More than 90\% of the cases of diabetes worldwide are classified as type 2 diabetes. The etiology of type 2 diabetes is complex and is associated with multiple defects, including impaired insulin secretion from pancreatic cells and insulin resistance in peripheral tissues, primarily skeletal muscle. Type 2 diabetes is a progressive disease, such that the initial development of hyperinsulinemia and skeletal muscle insulin resistance ultimately leads to a relative hypoinsulinemia and hyperglycemia. In order to regulate plasma glucose levels as close to normal as possible, dietary restrictions, exercise, and blood glucose-lowering agents are required (ADA, 2003).

The plant \textit{Stevia rebaudiana Bertoni} (Asteraceae) has been used as a tea for many years in the treatment of diabetes among Indians in Paraguay and Brazil. Stevia has no calories. It stimulates the release of insulin via a direct action on the pancreatic beta cells and normalizes the response to glucose, and considered to have the potential of becoming a new antidiabetic drug for use in type 2 diabetes (Jeppesen \textit{et al.}, 2000; Savita \textit{et al.}, 2004).

Gregersen \textit{et al.} (2004) hypothesize that supplementation with stevioside to a test meal causes reduction in blood glucose and tends to potentiate the insulin secretion in type 2 diabetic patients, indicating beneficial effects on the glucose metabolism. Stevioside may be advantageous in the treatment of type 2 diabetes. A 35\% reduction in blood glucose has also been observed in diabetic subjects after an oral intake of stevioside (Oveido \textit{et al.} 1979), which were demonstrated exerting a direct insulinotropic action in isolated mouse islets and in the clonal rat beta cell line (Jeppesen \textit{et al.}, 1996; 2000).
Research studies on the health benefits of consumption of stevioside are few and therefore, this investigation was undertaken to unravel if stevioside \textit{in vivo} exerts an antihyperglycaemic effect in diabetic rats compared with glibenclamide as a reference drug.

**MATERIALS AND METHODS**

**Stevioside used:** Stevioside crystalline, white powder, sweetness power 300 times as sucrose, procured from Stevia International Company for Agro-industry Product, in Egypt (SICAP) was used.

**Glucose and drugs used:** Glucose concentration kit was obtained from Biodiagnostic Co. (Dokki, Cairo, Egypt). Alloxan monohydrate and drug glibenclamide were obtained from El-Gomhoreya Co. (Cairo, Egypt).

**Experimental animals:** Twenty-four male albino Wister rats 180–220g body weight (BW) were obtained from Organization of Biological Products and Vaccines (Helwan Farm, Cairo, Egypt). The experimental rats were housed in screen-bottomed aluminum cages in rooms maintained on a 12/12 hour light/dark cycle during the experimental period according to Reynolds and Burger (1994).

**Experiment design:** On week from acclimation, the rats were divided into two parts. The first part represents Group 1 which consisted of 6 rats and was used as a control and received distilled water as vehicle. The second part contained 18 rats and injected intraperitoneally with freshly prepared solution of alloxan monohydrate at a dose of 150 mg kg\(^{-1}\) BW in three successive days. One week post injection, rats that showed fasting blood glucose more than 200 mg dl\(^{-1}\) were considered diabetic rats. These diabetic rats were divided into three groups (6 rats for each). Group 2 was kept as pathogenic diabetic as a control. Group 3 was given stevioside (500 mg kg\(^{-1}\) BW). Group 4 was given glibenclamide (600 µg kg\(^{-1}\) BW).

The estimated acceptable daily intake (ADI), which represents a level of daily intake that should result in no health hazard from a particular food additive, for rats was calculated as reported by Xilli \textit{et al.} (1992) from the ADI of stevioside in human (7.94 mg/kg/d) for a 100-fold safety factor. These doses of stevioside are only a small fraction of the LD\(_{50}\) for stevioside (15 g kg\(^{-1}\)) for the rat. The stevioside and the drug glibenclamide were given in aqueous solution daily using an intragastic tube for 3 weeks.

**Body weight and blood samples:** The changes in body weight were recorded weekly, at the end of the experimental period, the animals were sacrificed. Blood sample was collected in tubes containing heparin for the estimation of blood glucose.

**Enzymatic assay:** In this experiment the enzymatic determination of glucose in plasma was measured colorimetrically at 510 nm according to Trinder (1969).

**Statistical analysis:** All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980) and the statistical analysis system program (SAS, 1996) using Student t-test and factorial analysis.

**Histopathological examination:** Autopsy samples were taken from the pancreas of the sacrificed rats. According to the method of Banchroft \textit{et al.} (1996) samples were fixed in 10% formalin saline solution for 10 hours, and then washed in tap water for 12 hours. Serial alcohol concentrations were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at three micron thickness by slidge microtome. The obtained tissue sections were collected on
the glass slides and stained by hematoxylin and eosin stain for light microscopy.

RESULTS AND DISCUSSION

Body weight gain: The changes in the body weight in normal and experimental diabetic rats are represented in Figure-1. The body weights in the stevioside and glibenclamide treated groups 3 and 4 were increased significantly ($P < 0.001$) at the end of third week when compared with the diabetic control group 2.

![Figure-1](image.png)

**Figure-1.** Body weight gain in alloxan diabetic rats before and after oral treatment with Stevioside for 3 weeks. Groups: 1, normal; 2, diabetic control; 3 diabetic + Stevioside (500 mg kg$^{-1}$); 4, diabetic + glibenclamide (600 µg kg$^{-1}$). Values are given as mean ±S.D. from six rats in each group. Diabetic control was compared with normal, $#P < 0.001$. Experimental groups were compared with diabetic control $^{*}P < 0.001$.

**Glucose concentration in experimental rats:** The mean of blood glucose level in normal rats (Group 1) alone was stable throughout the experimental period. Conversely, in the alloxan-treated rats (Group 2) there was significant rise in blood glucose level, as compared to those of the control (Group 1). Although a significant antihyperglycemic effect was evident from the first week onwards, the decrease in blood glucose reached to the maximum after the third week compared to that of in the diabetic rats (Group 2). The percentage decreased in blood glucose at the end of the third week up to 34.52 and 51.86% in stevioside- and glibenclamide-treated rats of groups 3 and 4, respectively (Table 1 and Figure 2). The possible mechanism by which stevioside its hypoglycemic action in diabetic rats may be due to regulation of blood glucose levels by enhancing not only insulin secretion, but also insulin utilization in insulin-deficient rats as discussed by Chen et al. (2005).
Figure-2: Effect of stevioside on plasma glucose levels, Groups: 1, normal; 2, diabetic control; 3 diabetic + Stevioside (500 mg kg$^{-1}$); 4, diabetic + glibenclamide (600 µg kg$^{-1}$). Values are given as mean ± S.D. from six rats in each group.

Histopathological examination: Data in Figures-3 to 6 revealed the histopathological examination of semithin sections of pancreas of stevioside-treated rat cells stained with hematoxylin and eosin.

The control group 1 showed a normal histological structure of the endocrine portion as detected in the island of Langerhans cells as well as the acini of the exocrine portion (Figure 3). The normal observations of beta-cell structure of the control group are consistent with the observations reported by Jorns et al. (1997) for the same kind of cells.

Injection with alloxan (diabetic group 2) caused decline in the size and number of cells in the island of Langerhans with pyknosis in the nuclei (Figure 4). These changes are consistent with studies of Lanzen and Paten (1988) who reported that the injection of alloxan into experimental rats caused a selective specific necrosis of beta cells of the islets of Langerhans resulting in diabetes mellitus.

Treatment of alloxan-diabetic rats (Group 3) with stevioside showed that beta-cells were recovered normal size and number with pale eosinophilic cytoplasm (Figure 5). This result is in agreement with that of Jianguo et al. (2006), who stated that stevioside, a diterpene glycoside, has recently been shown to prevent glucotoxic effect by regulating Acetyl CoA carboxylase (ACC) activity and can alleviate impaired beta-cell function by regulating ACC activity.

Also the treatment of drug glibenclamide (Group 4) showed normal histological structure from the morphological structure of cells in islands of Langerhans (Figure 6).

The expected large increase in diabetes worldwide demands intensified investigations in new approaches for prevention and treatment of diabetes suitable even for low income populations in the developing countries. In this respect, this study paid an attention to use the stevioside as a new useful treatment in type 2 diabetes.

Figure-3: A micrograph shows a normal histological structure in the semithin-section in the pancreas cells of a rat in Group 1 (Control). (H & E, X-160).
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Figure-4: A micrograph shows a decline in the size and number of cells in the island of Langerhans with pyknosis in the nuclei the semithin-section in the pancreas cells of a rat in Group 2 (Control). (H & E, X-160).

Figure-5: A micrograph shows recovered beta-cells with normal size in the semithin-section in the pancreas cells of a rat in Group 3 (Control). (H & E, X-160).

Figure-6: A micrograph shows normal histological structure from the morphological structure of cells in the semithin-section in the pancreas cells of a rat in Group 4 (Control). (H & E, X-160).

REFERENCES


