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Immunohistochemical studies on the effect of *Aloe vera* on the pancreatic β-cells in neonatal streptozotocin-induced type-II diabetic rats

Sema Bolkent [1], Nuriye Akev [2*], Ayşe Can [2], Sehnaz Bolkent [3], Refiye Yanardag [4] & Alper Okyar [5]

1. Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University, 34098-Cerrahpasa, Istanbul, Turkey
2. Department of Biochemistry, Faculty of Pharmacy, Istanbul University, 34116-Beyazit, Istanbul, Turkey
3. Department of Biology, Faculty of Science, Istanbul University, 34134-Vezneciler, Istanbul, Turkey
4. Department of Chemistry, Faculty of Engineering, Istanbul University, 34320-Avcilar, Istanbul, Turkey
5. Department of Pharmacology, Faculty of Pharmacy, Istanbul University, 34116-Beyazit, Istanbul, Turkey

ABSTRACT

Aloe vera is used worldwide for several medical purposes as alternative medicine. There are positive and negative reports on the hypoglycaemic effects of this plant. From previous acute studies, *Aloe* leaf gel and pulp extracts lead to significant decreases in blood glucose in neonatal streptozotocin (n0-STZ)-treated type-II diabetic rats, whereas lowering of blood glucose during chronic treatment with the same extracts was statistically insignificant. Here we try to detect whether *Aloe* leaf gel and pulp extracts affect pancreatic β-cells. Using n0-STZ type-II diabetic rats, the immunoreactivity of β-cells of the islets of Langerhans did not differ among treatments of control, glibenclamide-, *Aloe vera* leaf pulp- and gel extract-treated rats. These results suggest that treatment of diabetic rats with *Aloe vera* gel or pulp or glibenclamide has no beneficial influence on the pancreatic β-cells in type II diabetes.

KEYWORDS: *Aloe vera*, type-II diabetes, pancreatic β-cells, immunohistochemistry

INTRODUCTION

Aloe vera L. Burm. f. or *Aloe barbadensis* Miller (Liliaceae) is native of North Africa and also cultivated in Turkey. This miraculous plant has been used in the traditional medicinal practices of many cultures for a host of curative purposes (Capasso *et al*. 1998, Vogler & Ernst 1999). Although oral hypoglycaemic agents are effective in controlling hyperglycaemia, they have prominent side effects (Rang *et al*. 1999). This leads to increasing demand for herbal products plant with antidiabetic activity and fewer side effects (Shane-Whorter 2001, Grover *et al*. 2002, Vetrichelvan & Jegadeesan 2002). In experimental diabetes, streptozotocin (STZ) causes selective degeneration of pancreatic β-cells thereby inhibiting insulin secretion. *A. vera* has also been shown to have antidiabetic and hypoglycaemic properties (Agarwal 1985, Hikino *et al*. 1986, Ghannam *et al*. 1986, Ajabnoor 1990, Beppu *et al*. 1993, Okyar *et al*. 2001a). It was postulated that hypoglycaemic effect of *A. vera* could be mediated through stimulation of synthesis and/or release of insulin from the β-cells of Langerhans (Ajabnoor 1990). However,

* Address for Correspondence

the mechanism of the reduction in mean glucose levels produced by *A. vera* has not been yet elucidated.

Some plant extracts shown to have hypoglycaemic activity have been tested for their activity on pancreatic β-cells: chard (*Beta vulgaris* L. var. *cicla*) (Bolkent *et al*. 2000), *Aegle marmelose* (Das *et al*. 1996), *Anastatica hierochuntica* (Rahmy & El-Ridi 2002) extracts were reported to improve the funtional state of pancreatic β-cells and thus reduce the histopathology provoked by STZ. In a recent study, prevention of the destruction of pancreatic islets by *Aloe arborescens* (Kidachi aloe) boiled-leaf-skin components was attributed to its free radical scavenging effect (Beppu *et al*. 2003).

To date, research on traditional antidiabetic plants has been especially focused on STZ-induced type-I diabetic rats. It has been assumed that, as an alternative to oral hypoglycaemic agents, herbal medicine can only be effective in type-II diabetes where pancreatic islets have not been not totally destroyed. This is the reason that neontal STZ-induced (n0-STZ) type-II diabetic rats (Bonner-Weir *et al*. 1981) were used in our investigation, and also why glibenclamide, a known hypoglycaemic agent, was used for comparison.

The aim of the present work was to evaluate the effects of *Aloe vera* leaf pulp and gel extracts as well as glibenclamide on the pancreatic β-cells morphology in STZ-induced type II diabetic rats

MATERIALS AND METHODS

Specimens of *Aloe vera* (L.) Burm. f. (Arabic: Sabr, Sabar, Saber; Turkish: Sarisabir) were collected from Kale (Demre) in Antalya in May 1993 (a voucher specimen was deposited in the Herbarium of Istanbul University, Faculty of Pharmacy. ISTE No. 65118), planted and cultivated in the greenhouse of the Faculty of Pharmacy. Fresh leaves of this cultivated plant were used in this study. Six large leaves were washed and cut from the middle, and the gel was separated. To prepare the leaf-pulp extract, the leaves without the gel (leaf pulp) were cut into small pieces (514 g) and homogenized with phosphate-buffered saline (PBS: pH 7; 600 ml) by means of a Moulinex Masterchief blender. The extract was kept at 4^{o}C overnight, then filtered through cloth and the filtrate centrifuged at 20,000 rpm for 30 min. at 2^{o}C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ). The green pellet was discarded and the clear yellow supernatant taken and lyophilized (using a Labconco apparatus). Thus 29 g of leaf-pulp extract was obtained. For use, leaf-pulp extract (7.5 %) was prepared by dissolving the powder in PBS and mixing thoroughly using a magnetic stirrer.

The leaf-gel extract was prepared as follows. The gel (400 g = 2.5 g dry matter) was homogenized in a Waring blender, then diluted with PBS (300 ml) and homogenized for a second time. The extract was kept at 4^{o}C overnight, and filtered through cloth. The clear filtrate was kept at –20^{o}C in small portions.

Glibenclamide (provided from Nobel; 5 mg) was suspended in 21 ml PBS; 4 ml propylene glycol was added and the mixture kept in an ultrasonic water bath (47.6 kHz.) for 45 min. until a homogenous suspension was obtained.

To obtain Type-II diabetic model rats, neonate Wistar pups were injected intraperitoneally on day 2 after birth with STZ (n0-STZ rats), at a rate of 100 mg/kg, freshly dissolved in cold citrate buffer (1 mM, pH 4.5) according to Bonner-Weir *et al*. (1981). This model of STZ-induced diabetes was reported by Portha *et al*. (1989) as potentially appropriate for investigations into diabetes pharmacotherapy. The animals were checked for the occurrence of diabetes after 6 weeks and the diabetics (fasting blood-glucose levels 104-170 mg/dl; mean 137 mg/dl, also in accordance with the type-II diabetic model) were used in the experiment when they were 2 months old (90-120 g weight). The animals were fed with laboratory pellets

and water *ad libitum*. Healthy (non-diabetic rats, 5 animals) were kept in the same conditions as the diabetic rats. Type-II diabetic rats were separated into 4 groups with 5-10 rats per group. Each group was given treatments as follows: Group I: (untreated diabetic control): PBS (6 ml/kg), Group II: *A. vera* leaf-pulp extract (500 mg/kg), Group III: *A. vera* leaf-gel extract (10 ml=63 mg/kg), Group IV: glibenclamide (1 mg/kg).

Each group of animals was treated daily over 14 days orally by means of a catheter under mild ether anaesthesia. Blood glucose levels (non-fasting) (reported in Turkish in Okyar *et al.* 2001b, and summarized here) were determined on the 7th and 14th days (using 32 µl blood taken from the tail vein in capillary tubes: Ringcaps Reflotron®) by the Reflotron® (Boehringer-Mannheim) glucose-oxidase method using the strips provided. The animals were sacrificed on the 15th day.

Tissue pieces taken from the pancreas of the rats on the 15th day were fixed by Bouin's solution and subsequently embedded in paraffin using routine procedures. The sections were dewaxed and rehydrated. After a washing step in PBS, they were immersed in 3 % H_2O_2 solution for 10 min. The sections were then kept in serum-blocking solution for 10 min. After incubation of the sections in primary monoclonal anti-insulin antibody (dilution 1:1000 µg/ml Sigma code I-2018) at 4 °C for one hour, they were immunostained according to the streptavidin-biotin-peroxidase method. A histostain SP kit (Zymed code 95-9943, San Francisco) was used for the immunohistochemistry. The localization of the antigen was indicated by a red colour, obtained with 3-amino-9-ethyl-carbozole (AEC) chromogen. Slides were counterstained with hematoxylin.

For controls for the specificity of antibody, the primary antibody step was omitted, or an inappropriate antibody (gastrin) was used. Control pancreas sections with (+) signals were used as a positive control.

RESULTS

Blood glucose levels of the rats are summarized in Table 1 (from Okyar *et al.* 2001b): treated diabetic animals did not differ from diabetic controls.

Table 1. Effect of *Aloe vera* leaf-pulp and gel extracts, and glibenclamide on blood glucose levels of type-II diabetic rats during chronic treatment (summarized from Okyar *et al*. 2001 b). There were no significant differences between the PBS-treated diabetic control and other treatments (using Student's *t*-test).

Group	**Blood glucose level mg %**		
	day 0	**day 7**	**day 14**
Untreated non-diabetic	98.1 ± 7.4	90.5 ± 5.4	87.0 ± 6.5
Untreated diabetic (PBS control)	185.4 ± 34.0	183.6 ± 43.0	182.0 ± 31.9
***Aloe* pulp**	202.7 ± 88.2	182.6 ± 68.4	229.0 ± 64.1
***Aloe* gel**	220.9 ± 63.5	205.6 ± 58.6	190.9 ± 67.7
Glibenclamide	199.6 ± 64.8	167.4 ± 41.8	164.5 ± 66.7

No immunohistochemical reaction was observed in negative control sections (Fig.1). In the control healthy group, immunoreactivity was observed in all β-cells as compared with the diabetic group (Fig 2). Only about half of the β-cells in the diabetic group gave positive signals

compared to the control group (Fig. 3). A decrease in the number of β-cells in this group was observed in comparison to the control group. The immunoreactivity of β-cells of the control diabetic group was not different from any of the treatmented groups: glibenclamide (Fig. 4), *Aloe vera* leaf gel (Fig. 5), or pulp extracts (Fig. 6).

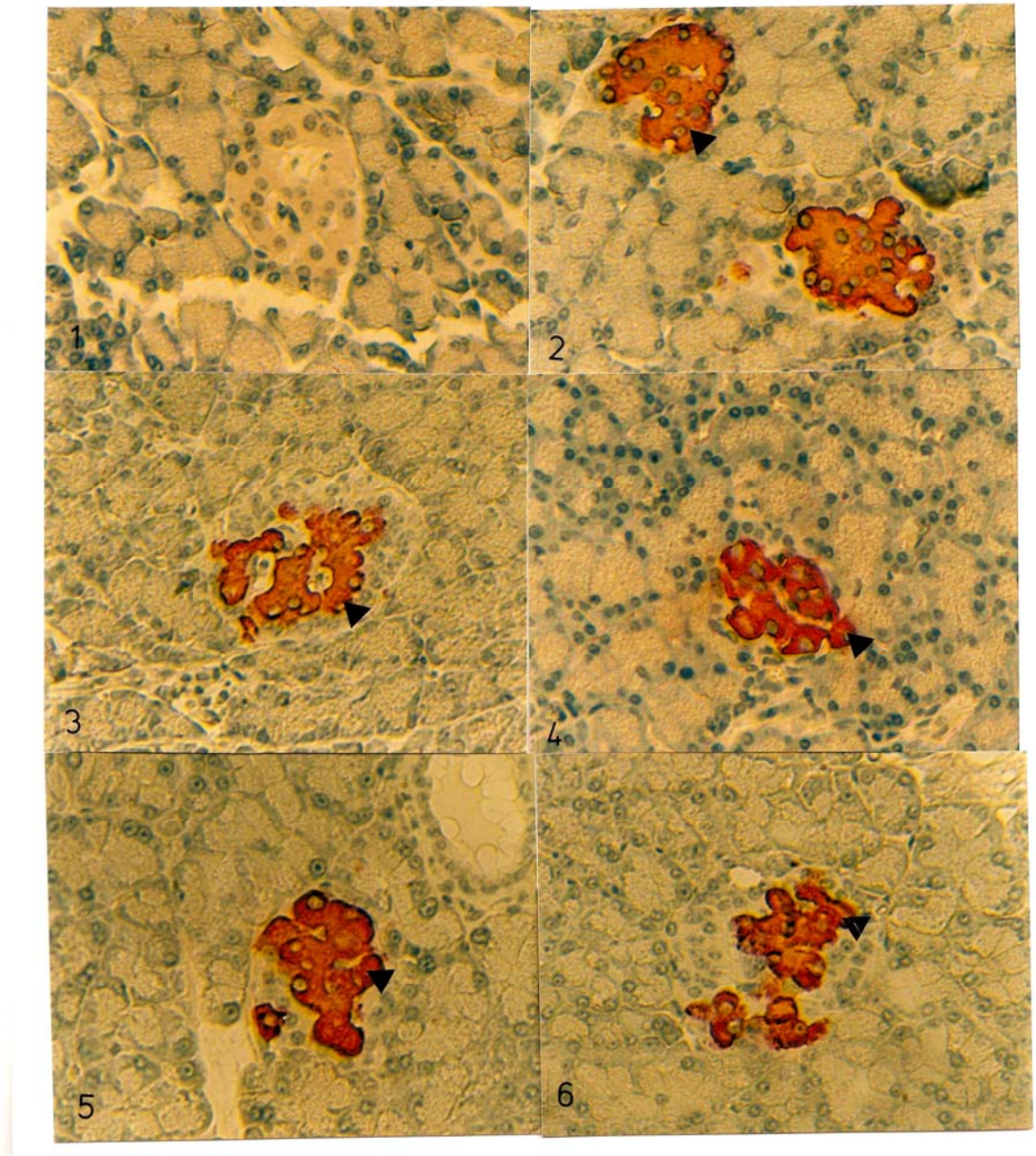

Fig. 1. Negative control (omission of primary antibody) section from control pancreatic islet. Counterstain haematoxylin. X 400.
Fig. 2. Insulin immunoreactivity (►) in Langerhans islets of the control rat. Streptavidin-Biotin-Peroxidase technique. Counterstain haematoxylin. X 400.
Fig. 3. Positive cells (►) in the pancreatic islet of the diabetic animal. Streptavidin-Biotin-Peroxidase technique. Counterstain haematoxylin. X 400.
Fig. 4. Insulin-producing cells (►) in Langerhans islets of the diabetic rat given glibenclamide. Streptavidin-Biotin-Peroxidase technique. Counterstain haematoxylin. X 400.
Fig. 5. Immunoreactive B-cells (►) in the pancreatic islet of the diabetic animal given *Aloe* gel extract. Streptavidin-Biotin-Peroxidase technique. Counterstain haematoxylin. X 400.
Fig. 6. Insulin immunoreactivity (►) in Langerhans islet of the diabetic rat given *Aloe* pulp extract. Streptavidin-Biotin-Peroxidase technique. Counterstain haematoxylin. X 400.

DISCUSSION

In recent years, various plant extracts have been claimed to be useful for the cure of diabetes mellitus (Palanichamy *et al*. 1988, Bailey & Day 1989, Ivorra *et al*. 1989, Neef *et al*. 1995, Perez *et al*. 1998, Shukla *et al*. 2000, Bolkent *et al.* 2000) but few of them have been tested for their effects on tissues of diabetic animals (Kern & Engerman 1987, Palanichamy *et al*. 1988, Parinandi *et al*. 1990, Bolkent *et al*. 2000): parsley extracts did not cause any morphological changes in pancreatic β-cells (Yanardag *et al.* 2003). Acute treatment with *Aloe* leaf pulp resulted in 30 and 34% decreases in blood sugar levels of n0-STZ-diabetic rats, after 2 and 3 hr of administration of the extract respectively (Okyar *et al*. 2001a), and 11 and 14% reductions in blood glucose levels 3 and 4 hr after administration of *Aloe* leaf-gel extract.

However these effects could not be repeated with the same extracts in chronic treatment (Okyar *et al*. 2001b). There were no significant differences in blood glucose levels between the groups given *Aloe* leaf-pulp and gel extracts compared to the control group given PBS, and there were no significant differences in blood sugar levels between *Aloe* extracts compared with the group given glibenclamide (see Table 1). Alongside claims of hypoglycaemic activity for *Aloe* extracts, (Agarwal 1985; Hikino *et al.* 1986; Ghannam *et al.* 1986; Ajabnoor 1990; Beppu *et al.* 1993; Okyar *et al.* 2001a), there are also reports of negative effects (Mossa 1985; Al-Awadi & Gumaa, 1987; Koo 1994; Chalaprawat 1997; Okyar *et al.* 2001b). These may be due to variation in the content and quality of natural drugs as well as to differences in the animals studied. Further studies with well-defined preparations or pure compounds are needed to eliminate the confusion which still exists about *Aloe* preparations. In the present study, the fact that neither glibenclamide nor *Aloe* extracts showed any stimulation of the pancreatic β-cells in type-II diabetic rats suggests that any decreases in blood glucose levels caused by *Aloe* extracts is not mediated by insulin release from β-cells, but by extra-pancreatic usage of glucose.

Thus we conclude that, contrary to reports in the literature (Rang *et al.* 1999¸ Chitra *et al.* 1998), treatment of diabetic rats with *Aloe vera* (gel and pulp) extracts or glibenclamide has no beneficial influence on the pancreas and thus may not be appropriate for the treatment of type-II diabetes in alternative medicine.

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