Original article

Effects of prolonged treatment with *Syzygium cumini* on the salivary glands of spontaneously diabetic mice

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Abstract

It is estimated that 190 million people worldwide have diabetes mellitus. Several attempts have been made to elucidate the deleterious effects of diabetes on various organ systems, as well as the reversal of these effects by treatment and/or diet. Thus, the objective of the present study was to analyze the effects of prolonged treatment with *Syzygium cumini* (Jambolan) sheet aqueous extract on the structure of cells responsible for secretory processes in the parotid and submandibular salivary glands of spontaneously diabetic mice. Ten female mice, including five diabetic Nod mice (group I) and five BALB/c mice (group II), were used. After characterization of the diabetic state, animals of group I received *Syzygium cumini* extract and group II animals received water *ad libitum*. After the experimental period, the salivary glands were collected from the animals for stereological analysis. The results showed structural alterations in the salivary glands of diabetic animals characterized by nuclear and cytoplasmic atrophy and the occurrence of inflammatory cells, as well as elevated glycemia levels. We conclude that no recovery of normal glycemia levels or glandular tissue structure occurs in diabetic animals even when treated with *Syzygium cumini* extract, a fact that might result in changes in the functional mechanisms of these organs.

Keywords: treatment, diabetes mellitus, salivary glands, structure.

1 Introduction

It is estimated that 190 million people worldwide have diabetes mellitus (RAVID and RACHMANI, 2005). The main characteristic of this disease is hyperglycemia, which is due to a deficiency in the utilization of carbohydrates resulting from gluconeogenesis and from abnormal insulin secretion or its effect on tissues, in addition to the exacerbated cellular accumulation of lipids and lipoproteins (ROBBINS, 1989; QUISSELL, REDMAN, BARZEN et al., 1994; CONGET, 2002). Diabetes type I manifests in approximately 10% of western diabetic patients (STEFAN, 1996) and its incidence has also been increasing among children (HUDGSON, OSSA, VELASCO et al., 2006). In South America, diabetes mellitus is one of the main public health problems and the fourth *causa mortis*. In addition, 50% of diabetic individuals do not know that they have the disease and 8% of the population aged 30 to 70 years currently expresses diabetes, corresponding to about 10 million people (MINISTÉRIO DA SAÚDE, 2007; PACE, NUNES and OCHOA-VIGO, 2003). In the United States, diabetes is responsible for 2% of death and is among the ten diseases that most kill in that country. In addition, diabetes is a debilitating disease characterized by various complications such as vascular, renal, ophthalmic and neurological deficiencies, as well as weight loss, digestive problems, abnormal wound healing processes and tissue repair, increased incidence of infections, and reproductive disorders (FUSHINI, 1980; HO, 1990; STEFAN, 1996; CAGNON, CAMARGO, ROSA et al., 2000; CALDEIRA, GARCIA, MINATEL et al., 2004).

Therefore, alternative therapies for the treatment of diabetes mellitus have been tested in several countries worldwide. Plants have been used as a source of drugs for the treatment of diabetes in developing countries, where the cost of conventional medicine represents a burden to the population. Some aspects of these plants, which are increasingly used for the treatment of different types of diseases, such as their therapeutic value, risk and toxicity, have raised scientific interest (BRAGANÇA, 1996; PEPATO, FOLGADO, KETTELHUT et al., 2001; OLIVEIRA, ENDRINGER, AMORIM et al., 2005).

Syzygium cumini, a plant of the family Myrtaceae commonly known in Brazil as "Jambolan", is popularly used for the treatment of diabetes mellitus (BRAGANÇA, 1996). The effect of this plant on blood glucose levels has been investigated in several studies. Soares, Costa and Cecim (2000) and Grover, Vats and Rathi (2000), using *Syzygium cumini* leaves and seeds, respectively, demonstrated the hypoglycemic action of the plant. Prince, Menon and Pari (1998), in addition to confirming the hypoglycemic effect of *Syzygium cumini*, also showed that the plant possesses an antioxidant effect, what can recuperate the tissues of the harmful effects caused by the diabetes.

Since diabetes mellitus is known to compromise salivary gland structure and mechanisms of salivation, which are relevant for oral and general health (EDGAR and O'MULLANE, 1996; CHAVEZ, TAYLOR, BORRELL et al., 2000; TAKAHASHI, SHINZATO and NAKAMURA, 2002; CALDEIRA, CAMILLI and CAGNON, 2005), thus, the aim of the present study was to analyze the therapeu-

tic effects of prolonged treatment with *Syzygium cumini* on cells responsible for secretory processes in the parotid and submandibular glands of spontaneously diabetic mice.

2 Material and methods

2.1 Animals and tissue preparation

Ten female mice obtained from the Animal Care Center (CEMIB/State University of Campinas Animal Care Centre), including five diabetic Nod mice (group I) and five BALB/c mice (group II), aged 16 weeks and weighing on average 22 g, were used in the present study. Group II animals served as negative controls of the confirmed diabetic group I mice.

Urine was monitored daily in animals of the two experimental groups for the evaluation of glucose levels (mg/dl) (Multistix 10 SG, Bayer, CA, USA). Fluid and solid intake was also measured daily over the experimental period. The body weight of the animals was determined at the beginning and at the end of the experiment.

After characterization of the diabetic state, animals of group I were treated with *Syzygium cumini* aqueous extract (200 mg.kg) and received chow *ad libitum* (OLIVEIRA, ENDRINGER, AMORIM et al., 2005) until the time of sacrifice for a maximum period of 20 days. To simulate the experimental conditions of the treated group, animals of group II were manipulated in the same manner and received solid Purina® (Purina, São Paulo, Brazil) chow pellets and water *ad libitum*. After the experimental period, the animals of the two groups were anesthetized with Francotar/Rompun (1:1) at a dose of 0.25 mL/100 g body weight and sacrificed (according to the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation, COBEA/CEP n. 021/07), and the parotid and submandibular glands were collected for stereological analysis under a light microscope. The salivary gland samples were fixed in Bouin's solution (picric acid solution), embedded in plastic resin (Araldite, Polysciences, Niles, IL, USA), and stained with hematoxylin and eosin (H&E) (BEHMER, TOLOSA, FREITAS-NETO, 1976). Photomicrographs were obtained with an Olympus V-CMAD 3 photomicroscope equipped with an Olympus C-7070 wide-zoom camera (Tokyo, Japan).

2.2 Preparation of Syzygium cumini sheet aqueous extracts

The plant material was dried, powdered and defatted with 70% ethanol. The extract was evaporated (40 °C) and one part of this crude extract was used in the experiment. This part was resuspended in water and successively extracted with dichloromethane and n-butanol. The fraction was evaporated, the aqueous fraction was lyophilized and the crude aqueous fraction of *Syzygium cumini* was used in the experiment (OLIVEIRA, ENDRINGER, AMORIM et al., 2005).

2.3 Stereology

Cytoplasmic and nuclear volumes were measured. Nuclear and cytoplasmic volumes were recorded as the average of 200 measurements per experimental group. Long and short axes were measured, and the mean nuclear volume was calculated considering the nuclei to be ellipsoid or spherical (WEIBEL, 1979).

2.4 Statistical analysis

Variations in body weight (g), nuclear volume (μm^3) and cytoplasmic volume (μm^3) were analyzed based on the mean profiles of the groups and on parametric and nonparametric variance, complemented by simultaneous confidence interval estimation, using the Tukey and Scheffé test (NORMAN and STREINER, 1994). The level of significance was set at 1% in all tests (MONTGOMERY, 1991).

3 Results

3.1 Glucose levels and treatment

Mean glucose levels were 0 to 120 mg/dl in group II and 570 mg/dl in group I. In addition, animals treated with *Syzygium cumini* extract did not recover normal glycemia levels, a fact that led to organ failure within a mean period of 20 days after confirmation of the effective diabetic state.

3.2 Body weight, and solid and fluid intake

Group I animals presented a significant loss of body weight when compared to group II, although solid and fluid intake was higher in group I (Table 1).

3.3 Parotid gland

In control mice (group II), serous acini consisting of columnar cells and a spherical and/or ellipsoid nucleus located in the basal region were observed (Figure 1a and Table 2). In group I, atrophic cells with a spherical nucleus also located in the basal region were observed after treatment with *Syzygium cumini*. Inflammatory cells were noted among the acini, as well as an enlarged intercellular space accompanied

Table 1. Body weight variation (final weigth-initial weigth) and fluid and solid intake in the experimental groups.

Groups	Weigth variation (g)	Fluid intake (mL)	Solid intake (g)
I	–1,2 ± 1,3*	168,8 ± 10,8*	50,2 ± 2,0*
II	2,8 ± 1,2	44,9 ± 4,3	32,6 ± 4,0
Statistical results	1,6 ($p < 0,01$)	126,1 ($p < 0,001$)	34,8 ($p < 0,001$)

Data are reported as mean ± S.D. *Indicate statistical differences at the 1% level of significance.

Table 2. Cytoplasmic (c) and Nuclear (n) volume in the different experimental groups.

Groups	Treatment	Parotid (n)	Parotid (c)	Submandibular (n)	Submandibular (c)
I	*Syzygium cumini* Extract	$37,1 \pm 1,4^a$	$92,7 \pm 11,3^a$	$28,9 \pm 2,3^a$	$84,2 \pm 8,6^a$
II	Water *ad libitum*	$312,0 \pm 87,1^b$	$445,2 \pm 125,7^b$	$227,1 \pm 9,6^b$	$356,3 \pm 19,9^b$

Data are reported as mean ± S.D. Different letters indicate statistical differences at the 1% level of significance.

by presence of adipocytes scattered throughout the stroma (Figure 1b, c and Table 2).

3.4 Submandibular gland

In control mice (group II), seromucous acini consisting of columnar cells with a basal spherical nucleus were noted (Figure 2a and Table 2). Group I animals presented atrophic cells with a spherical nucleus also located in the basal region. The space between acini was enlarged and inflammatory cells were observed (Figure 2b, c and Table 2).

4 Conclusion

The present study showed that animals treated with *Syzygium cumini* consumed a larger amount of chow and fluid than control animals. In addition, body weight loss was observed in diabetic animals treated with *Syzygium cumini*.

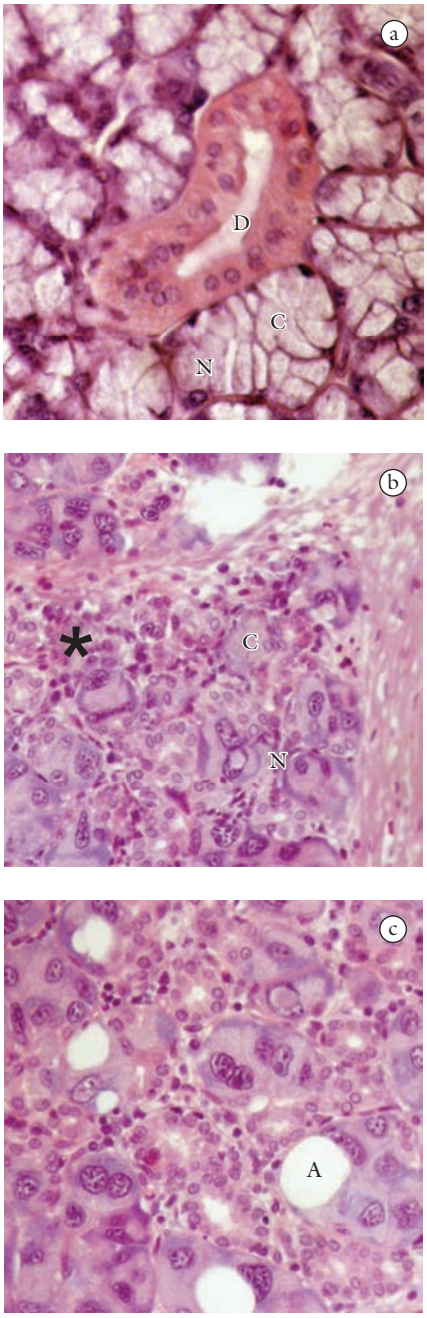

Figure 1. Photomicrograph of the parotid gland. A: Group II, observed secretory ducts (D), serous acini consisting of columnar cells (C) and basal nuclei (N). Magnification: 750×. H&E. B: Group I, treatment with *Syzygium cumini*, observed cellular atrophy (C), basal nuclei (N) and inflammatory cells (asterisco). Magnification: 250×. H&E. C: Group I, observed adipocytes (A) 750×. H&E.

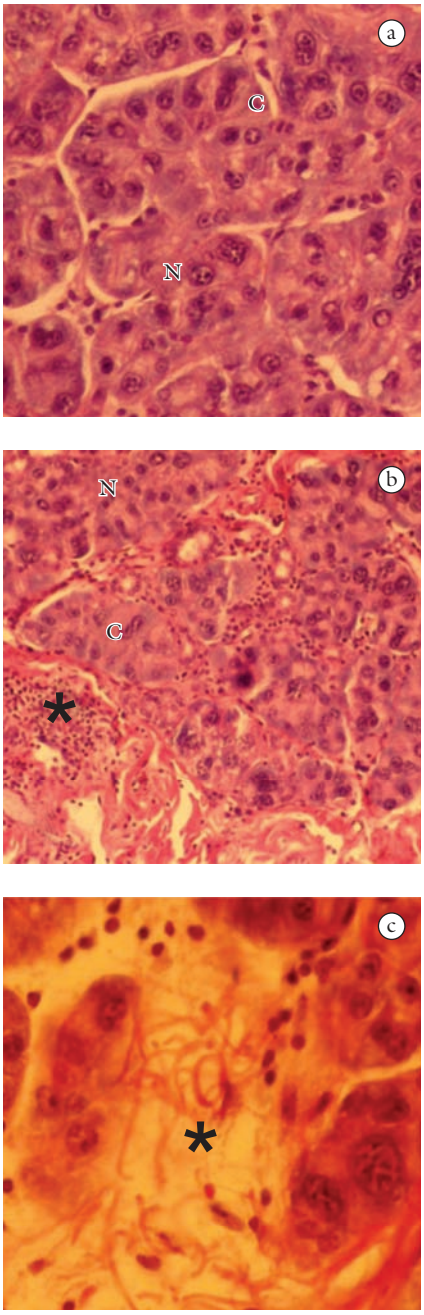

Figure 2. Photomicrograph of the submandibular gland. A: Group II, observed seromucous acini consisting of columnar cells (C) and basal nuclei (N). Magnification: 750×. H&E. B: Group I, observed cellular atrophy (C) basal nuclei (N), and inflammatory cells (asterisk). Magnification: 250×. H&E. C: Group I, observed, enlarged stromal space (asterisk). Magnification: 750×. H&E.

Diabetes mellitus causes metabolic disorders in different organ systems, including a loss of body weight and damage to different tissues (FUSHINI, 1980; MAKINO, KUNIMOTO, MURAOKA et al., 1980; HO, 1990; CAGNON, CAMARGO, ROSA et al., 2000; CONGET, 2002; CALDEIRA, CAMILLI and CAGNON, 2005; CALDEIRA and CAGNON, 2008). However, insulin treatment for 7 days has been shown to result in an increase of body weight in diabetic rats (ANDERSON, 1983; HE, SHI, WU et al., 2004), indicating that diabetes impairs body metabolism leading to a loss of body weight, which can be recovered by hypoglycemic treatments. However, treatment with *Syzygium cumini* was found to be unable to recover the body weight of diabetic animals.

With respect to glycemia, diabetic animals treated with the *Syzygium cumini* sheet extract maintained elevated glycemia levels, whereas normal levels were observed in control animals. Treated animals did not recover normal glycemia levels and died 20 days after beginning of treatment. In a study using Swiss Webster mice, a significant reduction in glucose levels was observed after 4 days of treatment with *Syzygium cumini*, demonstrating the hypoglycemic activity of this plant (VILLASEÑOR and LAMADRID, 2006). Several studies have also used Nod mice as a reliable diabetic model, with these animals presenting characteristics similar to those seen in human diabetic patients, a fact permitting clinical correlations (MORDES, BORTELE, BLANKENHORNEP et al., 2004; CALDEIRA, CAMILLI and CAGNON, 2005; CHAPARRO, KONIQSHOFER, BEILHACK et al., 2006; ROEP, 2007). According to Hu, Nakagawa, Purushotham et al. (1992), normal glycemic levels are close to 180 mg/dl in control animals, with levels higher than 300 mg/dl being considered to indicate an effective diabetic state. Pepato, Mori, Baviera et al. (2005) observed no significant improvement of glycemia levels or body fat in rats with chemically induced diabetes even after 15 days of treatment with *Syzygium cumini* extract obtained by decoction. In addition, another study investigating diabetic mice treated for 7 days with *Syzygium cumini* did not observe a reduction in glycemia levels in these animals (OLIVEIRA, ENDRINGER, AMORIM et al., 2005). However, studies have demonstrated the recovery of normal glucose levels in spontaneously diabetic mice treated with insulin for a period of 20 days, which remained alive during this period (CALDEIRA, CAMILLI and CAGNON, 2005). Thus, we conclude that the present findings confirm the diabetic state of the animals and characterize the inefficacy of treatment with *Syzygium cumini* in glycemic control.

The salivary glands analyzed presented tissue alterations in diabetic animals treated with the *Syzygium cumini* extract. The main alterations were cellular atrophy, an increase of fibrillary components and the presence of inflammatory cells. These alterations were more intense in submandibular salivary glands. Differences in the tissue response have been described in the literature, with the observation of no significant weight loss of the parotid glands in diabetic animals (ANDERSON and JOHNSON, 1981). Furthermore, different investigators have reported that, on average, 20 days after confirmation of the effective diabetic state both chemically induced diabetic rats and spontaneously diabetic mice (Nod) exhibit structural alterations in the salivary glands, such as reduced glandular weight and a decrease in secretory ducts and secretory granule density, as well as accumulation of lipid droplets in acini and ducts (ANDERSON, 1983; HIGH, SUTTON and HOPPER, 1985; HU, NAKAGAWA, PURUSHOTHAM et al., 1992; ANDERSON, SULEIMAN and GARRETT, 1994; LEIGH, SULEIMAN and GARRETT, 1994; SZCZEPANSKI, MEDNIEKS and HAND, 1998). Clinical and experimental studies have also identified functional alterations in salivary glands, as well as a severe inflammatory reaction (BLOCH, BUCHANAN, WOHL et al., 1965; GOILLOT, MUTIN and TOURAINE, 1991; HU, NAKAGAWA, PURUSHOTHAM et al., 1992; POZZILLI, SIGNORE, WILLIAMS et al., 1993; HUMPHREYS-BEHER, YAMACHIKA, YAMAMOTO et al., 1998; YAMANO, ATKINSON, BAUM et al., 1999; LÓPEZ, COLLOCA, PÁEZ et al., 2003). However, recovery of salivary secretion and of the tissue structure of salivary glands has been observed in diabetic rats submitted to glycemic treatment (ANDERSON, 1983; WATANABE, YAMAGISHI-WANG, KAWAGUCHI, 2001). Thus, based on the results we conclude that diabetes causes cell derangement in the parotid and submandibular salivary glands. In addition in the present study, on the other hand, the treatment with *Syzygium cumini* extract was ineffective in recovering general homeostasis and did not permit structural reorganization of the salivary glands in diabetic animals, a fact that might result in alterations in the functional mechanisms of these organs.

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