VIA MEDICA

ORIGINAL ARTICLE

Antidiabetic effects of *Eucalyptus globulus* on pancreatic islets: a stereological study

H. Mahmoudzadeh-Sagheb[1], Z. Heidari[1], M. Bokaeian[2], B. Moudi[3]

[1]Department of Histology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[2]Department of Laboratory Sciences, School of Paramedicine, Zahedan University of Medical Sciences, Zahedan, Iran
[3]Cell and Molecular Research Centre, Zahedan University of Medical Sciences, Zahan, Iran

The leaves of Eucalyptus globulus *(eucalyptus) are used for the treatment of diabetes mellitus in traditional medicine. The aim of this study was to evaluate the effects of eucalyptus on streptozotocin (STZ)-induced damage in pancreatic islands by stereological methods. Fifty mature normoglycaemic male Wistar rats, weighing 200–250 g, were selected and randomly divided into 5 groups (n = 10): control; STZ-induced diabetic (D) — by intraperitoneal injection of 60 mg/kg streptozotocin; treated control (TC); and treated diabetic (TD$_{1, 2}$), respectively, received 20 and 62.5 g/kg of eucalyptus in their diet, and 2.5 g/L aqueous extract of eucalyptus in their drinking water from one week after induction of diabetes. After four weeks of the experiment, stereological estimation of volume density and total volume of islets and beta cells, volume- -weighted mean islet volume, mass of the islets and pancreas, and total number of islets were carried out. Administration of eucalyptus significantly decreased the weight loss and increase of water and food intake in the treated diabetic groups in comparison to the STZ-induced diabetic (D) group. Volume density and total volume of islets, volume-weighted mean islet volume, mass of islets, and mass of pancreas of both treated diabetic groups were higher than the D group. In TD$_2$, these stereological parameters increased significantly compared to the D group (p < 0.001). Volume density and total volume of beta cells increased 21% and 65%, respectively, in the TD$_2$ group, but it was not statistically significant compared to the diabetic group (p > 0.05).*
The results suggested that Eucalyptus globulus *with a dose-dependent manner ameliorates diabetic states by partial restoration of pancreatic beta cells and repair of STZ-induced damage in rats. This study suggests a beneficial effect of eucalyptus in the treatment of diabetes.* (Folia Morphol 2010; 69, 2: 112–118)

Key words: diabetes mellitus, stereology, *Eucalyptus globules*, pancreatic islets, beta-cell

Address for correspondence: Dr. Zahra Heidari, PhD, Department of Histology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran, tel: +98 541 341 45 72, +98 915 141 42 92, fax: +98 541 341 45 61, e-mail: histology@ymail.com

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia. It may be due to a decrease in the synthesis of insulin (Type-I diabetes) or due to a decrease in the secretion of insulin from beta-cells of islets of the pancreas (Type-II diabetes) [25]. The increased glucose level generates glucose auto oxidation and auto oxidative glycosylation of proteins, which leads to oxidative stress by increasing the reactive oxygen species [24]. Diabetes mellitus produces various dysfunctions in the body, including vascular disorder, retinopathy, cardiomyopathy, altered immune functions, changes in the intestinal function, peripheral neuropathy, and dysfunction of the central nervous system [25]. Diabetes treatment is essential for preventing or at least delaying the onset of these complications [29]. For many years, a diet rich in vegetables and fruit has been recognized as protecting against chronic diseases such as diabetes. It seems that treatment with herbal drugs can protect pancreatic beta-cells and reduce fluctuations in glucose levels [17]. Although present therapy for diabetes mellitus relies on an arsenal of drugs, diabetes therapy also revolves around dietary measures including the use of traditional antihyperglycaemic plants [1, 19, 22, 28] such as *Eucalyptus globulus*. *Eucalyptus globulus* Labill (Myrtaceae) is a fast-growing, evergreen tree, native to Tasmania and south-eastern Australia. It grows best in Mediterranean climates (cool, wet winters and dry, warm summers). It was first grown in Iran about half century ago, and the plantation of many of such species in the north and south had very satisfactory results [23]. It is widely distributed throughout the Sistan and Baluchestan provinces. The trees flower and the fruits ripen throughout the year. It grows in dense monocultures. Juvenile leaves are about 6 to 15 cm long and are covered with a blue-grey, waxy bloom. The mature leaves are narrow, sickle-shaped, and dark shining green and range from 15 to 35 cm in length. The flower clusters develop within an envelope formed by two bracteoles which split and are shed, exposing the flower buds. The fruit is a woody capsule 6–25 mm in diameter. Numerous small seeds (3–6) are shed through valves which open on the top of the fruit. It produces roots throughout the soil profile, rooting several feet deep in some soils [23]. *Eucalyptus globulus* is traditionally used to treat diabetes in South America, Africa [7], and Iran [8]. Its medicinal uses are as an antiseptic and deodorant, to treat hoarseness, coughs, whooping coughs, asthma and bron-

chitis, coryza, dysentery, diabetes, fevers and colds, malaria, rhinitis, and tuberculosis, and for washing and cleaning wounds [7]. Leaves from *Eucalyptus globulus* are reported to contain a high content of eucalyptol (cineol) together with rutin, terpineol, sesquiterpene, alcohols, aliphatic aldehydes, isoamyl alcohol, ethanol, terpenes, and tannins [7, 8]. A study in streptozotocin (STZ)-diabetic mice confirmed the antihyperglycaemic effectiveness of *Eucalyptus globulus*. The possibility exists that dietary administration of eucalyptus was associated with the protection or regeneration of pancreatic beta-cells as insulin producing cells of islets following STZ-induced diabetes [9]. The available literature presents no data relating to the effect of a *Eucalyptus globulus* supplemented diet and drinking water on quantitative parameters of pancreatic islets in STZ-induced diabetic rats. Therefore, in this study the effects of *Eucalyptus globulus* on pancreatic islets and beta cells in STZ-induced diabetic rats were investigated by stereological methods.

MATERIAL AND METHODS

Plant material

Eucalyptus globulus Labill leaves were collected fresh from a garden in Zahedan University of Medical Sciences (ZUMS). The plant was identified and verified at the Herbarium of Botany Directorate in Sistan and Baluchestan University, Zahedan, Iran. A voucher specimen was deposited in the Botany Department of Sistan and Baluchestan University.

Preparation of eucalyptus-incorporated diet and aqueous extract of *Eucalyptus globulus*

The leaves were homogenized to a fine powder and stored at room temperature (20 ± 2°C) until use. For the animals, eucalyptus was incorporated into a powdered diet, thoroughly mixed, distilled water was added, and it was mixed into a stiff paste. The mixture was then pelleted and dried at 45°C. The control diet was prepared by the same method to ensure there were no end differences in vitamin and mineral content due to the drying process [9].

Aqueous extract of eucalyptus (AEE) was prepared by 15 minutes decoction of the powdered material, as described by Gray and Flatt [9].

Animals

The study was performed on 50 matured normoglycaemic male albino rats (Rattus norvegicus) of Wistar strain (ZUMS animal lab, Zahedan, Iran),

113

weighing 200–250 g, separately housed in cages (one rat per cage), with free access to water and food. The animals were maintained in a room at 23 ± 2°C with a fixed 12-hour artificial light period, and the air was adequately recycled. All animals were fed with standard rodent diet with the following composition (w/w): 20% protein, 3% fat, 2% fibre, 6% minerals, and 69% starch and vitamin supplements (Pasture institute, Iran). All animals received humane care as outlined in the guide for the care and use of laboratory animals. The Ethical Committee of Zahedan University of Medical Sciences approved this study.

Experimental design

Fifty rats were divided into the five following groups (n = 10):
(I) Control group (C): rats of this group received rodent diet and tap water. After one week they received intraperitoneal vehicle (0.15 M NaCl with 100 mM sodium citrate buffer).
(II) Diabetic group (D): in this group diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg of body weight in 0.15 M NaCl with 100 mM sodium citrate buffer, pH 4.5).
(III) Treated control group (TC): healthy rats received eucalyptus supplemented diet and drinking water. Eucalyptus was incorporated into the diet (62.5 g/kg) and drinking water (2.5 g/L) [9].
(IV and V) Treated diabetic groups ($TD_{1, 2}$): these groups received, respectively, 20 and 62.5 g/kg eucalyptus in the diet, and 2.5 g/L AEE in drinking water, from one week after induction of diabetes by streptozotocin.

The eucalyptus treatment began one week after induction of diabetes and lasted for four weeks, and then the rats were killed. Food and fluid intake of all groups were measured daily. Body weight and blood glucose were measured every week.

Glucose measurement

Blood samples were collected from the tail vein. Blood glucose levels were measured by standard method of oxidase-peroxidase paired enzyme adapted for a RA 1000 analyzer (Technicon, USA).

Preparation of tissues

At the end of the experiment, and after overnight fasting, all animals were sacrificed. The pancreases were quickly removed, placed in cold saline solution and trimmed of adipose tissue, and weighed, and volumes were measured using immersion method

and fixed in modified Lillie's solution for one week at room temperature. Using the orientator method, 10–12 isotropic uniform random sections were obtained. Briefly, each pancreas was placed on a circle that was divided into 36 equal sectors, and was sectioned along the line bearing a randomly selected number. The sectioned surface of the bar was placed on the 0–0 direction of the circle with 97 unequal sinus-weighted divisions and the second cut was done. After routine histological processing, the new surface was embedded in paraffin, sectioned (5 μm thickness) [6, 10, 13], and stained by modified aldehyde fuchsin histochemical method [2].

Stereological study

Total number of islets, volume-weighted mean islet volume, pancreatic islet and beta cell volume density and total volume

Two sets of primary and reference sections through the pancreas were sampled using systematic uniform random sampling (SURS) [3, 20].

The total number of islets was determined as previously described by the so-called physical fractionation method [5, 10] using this formula:

$$estN_{isl} = \frac{N_{sect\,(p–p)}}{N_{sect\,(p–r)}} \times \frac{\Delta X \times \Delta Y}{A_{frame}} \times \Sigma Q_{isl}$$

where $N_{sect\,(p–p)}$ is the number of sections between the primary sections, and $N_{sect\,(p–r)}$ is the number of sections between a primary section and the corresponding reference section. ΔX is the step length in the x direction, ΔY is the step length in the y direction, A_{frame} is the area of the sampling frame corrected for magnification, and ΣQ_{isl} is the total number of islets counted (sampled in the primary section but absent in the reference section) from one pancreas [5].

To determine the volume-weighted mean islet volume, five to seven systematic random fields were sampled per primary section. At a final magnification of 400×, a grid of standard points and a set of parallel lines of random orientation were superimposed randomly onto the image and the volume-weighted mean islet volume was estimated using the point sampled intercepts method [11–13].

Data were entered into a result sheet and the volume-weighted mean islet volume was estimated by this formula:

$$estv_v = \frac{\pi}{3} \cdot l_3^0 \cdot F$$

where, Vv is the volume-weighted mean islets volume, l_3^0 is the mean of the cubed measured intercepts length, and *F* is:

$$\left(\frac{1}{\text{magnification}}\right)^3 \text{ [26].}$$

In order to estimate the volume density of islets, ten to twelve sections were sampled from each gland by systematic uniform random sampling [13]. A BH2--Olympus light microscope with a projecting arm was used. On each sampled section, five to seven fields were selected in a systematic random manner. A test system was superimposed on these fields, and points hitting the various components of the gland were counted. Then an estimate of the volume density, Vv, of the components in the reference space was obtained using:

$$\text{estv}_v = \frac{P_{(part)}}{P_{(ref)}}$$

where $P_{(part)}$ and $P_{(ref)}$ are the number of test points falling in all structure profiles and in the reference space, respectively [10, 11, 13].

In order to estimate the absolute volume of a part, the volume density of that part is multiplied by the reference volume [13].

Total mass of islets, and pancreas

Total islet mass was determined as previously described [11–13]. A point-counting grid with 99 points, 1 of them encircled (the unit point), was attached to the table, and for each pancreas the total number of grid points that hit islets and the total number of unit points that hit the pancreas were counted [5]. The total islet mass for each pancreas was then estimated by:

$$\text{estM}_{isl} = \frac{P_{isl}}{99 \times (P_{tis})} \times M_{tis}$$

where M_{isl} is the total islet mass, P_{isl} is the total number of grid points that hit islets in all explored sections from one pancreas, P_{tis} is the number of unit points that hit the removed tissue, and M_{tis} is the wet weight of it.

The mass of pancreas was estimated using this formula:

$$\text{estM}_{pan} = \frac{P_{pan}}{P_{tis}} \times M_{tis}$$

where P_{pan} is the number of unit points that hit the pancreatic tissue.

Statistical analysis

Data are presented as the means ± SE for each investigated parameter. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons were used to compare differences between the experimental groups. The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS 11 for Windows software system.

RESULTS

Effects of eucalyptus treatment on physical status, blood glucose level, and islet histology

Table 1 shows body weight, food and fluid intake, and fasting blood glucose levels in the control and experimental groups. Diabetic rats showed significant ($p < 0.001$) weight loss and increase of water and food intake compared with control rats at the end of the treatment period. Administration of eucalyptus significantly decreased the weight loss and water and food intake in eucalyptus treated-diabetic rats ($TD_{1, 2}$) compared to untreated-diabetic rats. After STZ injection, the blood glucose levels of the D and $TD_{1, 2}$ groups significantly increased ($p < 0.001$) in comparison to controls. Treatment of $TD_{1, 2}$ rats with eucalyptus extract displayed a significant, hypoglycaemic effect in STZ-induced diabetic rats in comparison to the corresponding D rats. No significant changes in blood glucose levels were observed after administration of eucalyptus in normally treated (TC) rats (Table 1).

Table 1. Changes in body weight, food and fluid intake, and serum glucose in experimental groups (n = 10), at the end of the experiment

Groups	C	TC	D	TD_1	TD_2
Final body weight [g]	335.0 ± 4.1	326.6 ± 6.3	176.4 ± 4.1[a]	237.6 ± 7.8[b]	280.2 ± 25.7[b]
Food intake [g/day]	23.1 ± 0.7	21.8 ± 1.5	59.4 ± 1.1[a]	33.6 ± 3.2[b]	27.7 ± 0.6[b]
Fluid intake [mL/day]	51.2 ± 2.0	54.8 ± 2.7	298.5 ± 3.7[a]	123.7 ± 5.6[b]	58.8 ± 8.7[b]
Serum glucose [mL/dL]	91.1 ± 2.7	87.9 ± 4.2[a]	281.4 ± 5.5[a]	203.3 ± 3.2[b]	117.8 ± 5.3[b]

The values represent the mean ± SE; C — control; D — diabetic; TC — treated control; $TD_{1,2}$ — treated diabetic received respectively with 20 g/kg and 62.5 g/kg eucalyptus in diet; + 2.5 g/L aqueous extract of eucalyptus in drinking water; [a]$p < 0.001$ compared to control group and [b]$p < 0.001$ compared to diabetic groups

Pancreas histological sections in control and treated control animals possessed normal islets with clusters of purple granulated beta cells. In diabetic rats, STZ caused atrophy of the islets and degenerative changes of beta cells. Eucalyptus treatment partially restored cellular degeneration of beta cells of islets in treated diabetic rats.

Effects of eucalyptus treatment on stereological parameters

As indicated in Table 2, diabetes caused a significant reduction of pancreatic mass (16.65%), islet mass, beta cell mass, volume density of islet/pancreas, total islet volume, volume density of beta cells/ /islets, total beta cell volume, and volume-weighted mean islet volume compared to the control group ($p < 0.001$). Treatment with eucalyptus compensates these diabetic changes. In the $TD_{1,2}$ group, in comparison with the D group, there was, respectively, a 3.4% and 6.3% increase in pancreatic mass, which in the TD_2 group was statistically significant ($p < 0.05$).

In the $TD_{1,2}$ groups, compared to the D group, islet mass increased by 22.9% and 39%, respectively, volume density of islet/ pancreas increased by 12% and 32%, respectively, total islet volume increased by 13% and 34%, respectively, and volume-weighted mean islet volume increased by 17% and 22%, respectively. These parameters in TD_2 were statistically significant from the D group ($p < 0.05$). Volume density of beta cells/islets increased 5% and 21%, and total beta cell volume increased 20% and

65% in TD_1 and TD_2 groups, respectively, but these differences were not statistically significant from the D group ($p > 0.05$). The total number of islets, pancreas wet weight, and volume did not show any significant changes between these groups ($p > 0.05$).

DISCUSSION

The present study showed that the treatment of diabetic rats with *Eucalyptus globulus* for four weeks compensated the diabetic state and significantly reduced blood glucose levels in comparison to the diabetic rats.

Streptozotocin injection results in diabetes mellitus, which may be due to selective destruction of beta cells [18]. STZ-induced diabetes is characterized by weight loss, increase of water and food intake, and high blood glucose levels [28]. In treated-diabetic rats ($TD_{1,2}$), body weight significantly increased, and water and food intake significantly decreased, compared to the D group.

In agreement with our results in TC group, Jouad et al. [16] showed that single and repeated oral administration of AEE exhibited a significant, dose-dependent hypoglycaemic effect in STZ diabetic rats. He also showed that in rats with a normal functioning pancreas, no significant changes in blood glucose levels were observed after administration of *Eucalyptus globulus* leaf extract. Ismail [15] showed that oral administration of AEE in fasted rats exhibited a significant, dose-dependent hypoglycaemic effect, which represents an effective anti-hyperglycaemic dietary supplement for the treatment of

Table 2. Pancreas wet weight, mass and volume and stereological parameters of the islets and beta cells in experimental groups

Parameter	C (n = 10)	TC (n = 10)	D (n = 10)	TD_1 (n = 10)	TD_2 (n = 10)
Pancreas wet weight [g]	1.12 ± 0.02	1.11 ± 0.02	1.06 ± 0.02	1.07 ± 0.02	1.07 ± 0.02
Pancreatic mass [mg]	560.5 ± 6.2	551.3 ± 7.6	467.2 ± 6.6^a	483.0 ± 8.2^a	$496.7 \pm 6^{a,\,b}$
Pancreas volume [$\times 10^3$ mm^3]	2.01 ± 0.03	2.02 ± 0.05	1.95 ± 0.04	1.96 ± 0.05	1.97 ± 0.05
Islets mass [mg]	64.5 ± 1.8	64.6 ± 1.5	24.9 ± 1.5^a	30.6 ± 1.6^a	$34.6 \pm 2^{a,\,b}$
Volume density islets/pancreas (%)	28.4 ± 0.8	28.7 ± 1	13.9 ± 0.8^a	15.6 ± 1.2^a	18.4 ± 1.5^b
Total volume of islets [mm^3]	572 ± 21	578 ± 16	271 ± 17^a	305 ± 25^a	$363 \pm 31^{a,\,b}$
Volume density beta cells/islet (%)	73.2 ± 1.8	72.5 ± 1.5	31.7 ± 2.3^a	33.3 ± 1.9^a	38.4 ± 2^a
Total volume of beta cells [mm^3]	41.9 ± 1.9	42.0 ± 1.7	8.5 ± 0.8^a	10.2 ± 1.1^a	14.0 ± 1.5^a
Volume weighted mean islet volume [$\times 10^6\ \mu m^3$]	4.11 ± 0.07	4.04 ± 0.1	1.95 ± 0.07^a	2.28 ± 0.12^a	$2.37 \pm 0.13^{a,\,b}$
Total islet number [$\times 10^3$]	23.0 ± 1.5	23.2 ± 1.3	21.9 ± 0.8	22.5 ± 0.7	22.7 ± 0.5

Values are mean $\pm$ SE; "n" stands for number of rats in each group. CEs for point counting in each measurement are less than 0.05; C — control; D — diabetic; TC — treated control; TD_1 and TD_2 — diabetic rats treated respectively with 20 g/kg and 62.5 g/kg eucalyptus in diet; + 2.5 g/L aqueous extract of eucalyptus in drinking water; [a]$p < 0.05$ compared to control group and [b]$p < 0.05$ compared to diabetic groups

116

diabetes mellitus. It has been reported that *Eucalyptus globulus* extract contains high levels of manganese [21]. Manganese chloride can exert a hypoglycaemic action [9]. Thus the antidiabetic action of this plant can be attributed, at least in part, to the high concentration of manganese. Gray and Flatt [9] suggested that eucalyptus probably acted by modulating insulin secretion and/or insulin action. Another possibility that they proposed was that eucalyptus can regenerate beta cells or protect them from STZ--induced damage. The present investigation focused on this view by using stereological methods. Following eucalyptus administration, an increase in pancreas and islet mass, volume-weighted mean islet volume, volume density of islets, and total volume of islets were found in the $TD_{1,2}$ groups compared to the D group. These parameters in the TD_2 group were statistically significant in comparison with the diabetic group. This showed a dose-dependent effect of eucalyptus on pancreatic islets. In this study, the total number of islets was not significantly different between the experimental groups. Our previous work on beta cell protective effects of sodium tungstate also showed that the number of islets was almost constant in all groups [12]. Possibly, the architecture of the islets is complex to the extent that it only allows new islets to be formed during the formation, growth, or regeneration of the pancreas [4]. The number of pancreatic islets seems especially to be under tight genetic control [5], and STZ evokes selective deleterious changes in beta cells but it cannot cause complete disappearance of the entire islet [27], thus there was not any significant reduction in the number of islets in the D rats. It can be conclude that the increase in total volume of islets in the eucalyptus-treated rats was caused entirely by islet size due to partial restoration of beta cells after STZ-induced damage. Skau et al. [26] reported also that the increase in the total islet volume during physiological growth in rats is attributable to islet hypertrophy with no contribution from islet hyperplasia (increase in the total number of islets). In the TD_2 group, the volume density of beta cells/islets and total volume of beta cells increased by 21% and 65%, respectively, in comparison to the D group. This increase shows a dose-dependent partial recovery of beta cell population. New beta cells could be derived from intra-islet stem/progenitor cells [26]. Inuwa and El Mardi [14] showed that the mass of pancreatic islets is dynamic and can be modified in response to changes in metabolic demand to maintain normoglycaemia. Islet mass depends on, among other factors, the changes in beta and alpha cell formation, individual cell size, and rate of cell death. The balance between these elements determines whether islet mass is increased, remains stable, or is reduced. In the $TD_{1,2}$ groups, the increase in pancreatic islet mass was probably due to an increase in the mass of its cells, especially beta cells, because they are the predominant cell type in the islet, and changes in their biological dynamics are the most important factors that determine islet morphology [14]. Gray and Flatt [9] indicated that eucalyptus is likely act by modulating insulin secretion and//or action. Studies using clonal pancreatic beta cells showed that AEE exerted a dose-dependent stimulatory effect on insulin secretion. Jouad et al. [16] showed that the repeated oral administration of AEE significantly increased the basal plasma insulin concentrations. The levels of insulin were not examined in our study. However, it seems that partial restoration of pancreatic beta cells, and stimulation of all functional beta cells following eucalyptus treatment, could be the reasons for the treatment of the diabetic state of the rats in this study. The second aspect requires further study. It seems that further investigation with higher doses of eucalyptus, and/or longer periods of treatment will be necessary for more effective results. The chemical nature of the antihyperglycaemic constituent(s) of eucalyptus also remain(s) to be established.

CONCLUSIONS

It can be concluded that eucalyptus in a dose--dependent manner could compensate STZ-induced cell damage of pancreatic beta cells. Thus, it may be an effective antihyperglycaemic dietary supplement for the treatment of diabetes and a potential source for the discovery of new orally active agent(s) for future diabetes therapy. Further comprehensive chemical and pharmacological investigations with isolated active principles of the plant may shed more light on the use of eucalyptus for antidiabetic activity in diabetic animals and patients.

ACKNOWLEDGMENTS

REFERENCES

1. Arise RO, Malomo SO, Adebayo JO, Igunnu A (2009) Effects of aqueous extract of *Eucalyptus globulus* on

lipid peroxidation and selected enzymes of rat liver. J Med Plants Res, 3: 77–81.

2. Bancroft JD, Gamble M (2002) Theory and practice of histological technique. 5th Ed. Churchil Livingston, London, pp. 556–557.

3. Barbera A, Fernardez-Averez J, Truce A, Gomis R, Guinovart JJ (1997) Effects of tungstate in neontally streptozotocin-induced diabetic rats: mechanism leading to normalization of glycemia. Diabetologia, 40: 143–149.

4. Bock T, Kyhnel A, Pakkenberg B, Buschard K (2003) The postnatal growth of the beta-cell mass in pigs. J Endocrinolol, 179: 245–252.

5. Bock T, Pakkenberg B, Buschard K (2005) Genetic background determines the size and structure of the endocrine pancreas. Diabetes, 54: 133–137.

6. Cruz-Orive LM, Weibel ER (1990) Recent stereological methods for cell biology: a brief survey. Am J Physiol, 258: L148–L156.

7. Duke JH (1985) CRC Handbook of medical herbs. CRC Press, Boca Raton, Florida, p. 550.

8. Eidi A, Eidi M, Givianrad MH, Abaspour N (2009) Hypolipidemic effects of alcoholic extract of eucalyptus (*Eucalyptus globulus* Labill.) leaves on diabetic and non--diabetic rats. Iran J Diab Lipid Dis, 8: 105–112.

9. Gray AM, Flatt PR (1998) Antihyperglycemic actions of *Eucalyptus globulus* (Eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. J Nutr, 128: 2319–2323.

10. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard. JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS, 96: 379–394.

11. Gundersen HJG, Jensen EB (1985) Stereological estimation of the volume-weighted mean volume of arbitrary particles observed on random sections. J Microsc, 138: 127–142.

12. Heidari Z, Mahmoudzadeh-Sagheb HR, Moudi B (2008) A quantitative study of sodium tungstate protective effect on pancreatic beta cells in streptozotocin-induced diabetic rats. Micron, 39: 1300–1305.

13. Howard CV, Reed MG (1998) Unbiased stereology: three-dimensional measurement in microscopy. 1st Ed. Biosscientific Publishers, Oxford, pp. 55–68.

14. Inuwa IM, El Mardi AS (2005) Correlation between volume fraction and volume weighted mean volume, and between total number and total mass of islets in postweaning and young Wistar rats. J Anat, 206: 185–192.

15. Ismail SM (2007) The effect of aqueous extract of the leaves of eucalyptus globules on the blood glucose level in fasted rats. Iran J Pharmacol Therap, 6: 185–186.

16. Jouad H, Maghrani M, El Hassani RA, Eddouks M (2004) Hypoglycemic activity of aqueous extract of Eucalyptus globulus in normal and streptozotocin-induced diabetic rats. J Herbs, Spices Med Plants, 10: 19–28.

17. Kang YJ, Jung UJ, Lee MK, Kim HJ, Jeon SM, Park YB, Chung HG, Baek NI, Lee KT, Jeong TS, Choi MS (2008) Eupatilin, isolated from Artemisia princeps Pampanini, enhances hepatic glucose metabolism and pancreatic beta-cell function in type 2 diabetic mice. Diab Res Clin Pract, 82: 25–32.

18. Kavalali G, Tuncel H, Göksel S, Hatemi HH (2003) Hypoglycemic activity of *Urtica pilulifera* in streptozotocin-diabetic rats. J Ethnopharmacol, 84: 241–245.

19. Mallick C, Maiti R, Ghosh D (2006) Comparative study on antihyperglycemic and antihyperlipidemic effects of separate and composite extract of seed of *Eugenia jambolana* and root of *Musa paradisiaca* in Streptozotocin-induced diabetic male albino rat. Iran J Pharmacol Therap, 5: 27–33.

20. Partida-Hernandez G, Arrola F, Fenton B, Cabeza M, Roman-Ramos R (2006) Effect of zinc replacement on lipid and lipoproteins in type 2-diabetic patients. Biomed Pharmacol, 60: 161–168.

21. Pinto G, Coutinho J, Araujo C, Neves L, Santos C (2007) Importance of media mineral composition on the induction of somatic embryogenesis in Eucalyptus globulus labill. Boletin de CIDEU, 3: 83–90.

22. Rao NK, Nammi S (2006) Antidiabetic and renoprotective effects of the chloroform extract of Terminalia chebula Retz seeds in streptozotocin-induced diabetic rats. BMC Complementary Altern Med, 6: 17.

23. Sabet H (1994) Forests, trees, and shrubs of Iran [In Persian]. Yazd University Publication, Yazd, Iran, pp. 334–337.

24. Shabeer J, Srivastava RS, Singh SK (2009) Antidiabetic and antioxidant effect of various fractions of *Phyllanthus simplex* in alloxan diabetic rats. J Ethnopharmacol, 124: 34–38.

25. Sreemantula S, Kilari EK, Vardhan VA, Rajasekhar Jaladi R (2005) Influence of antioxidant (L-ascorbic acid) on tolbutamide induced hypoglycaemia/antihyperglycaemia in normal and diabetic rats. BMC Endocrine Disorders, 5: 2.

26. Skau M, Pakkenberg B, Buschard K, Bock T (2001) Linear correlation between the total islet mass and the volume-weighted mean islet volume. Diabetes, 50: 1763–1770.

27. Takasu N, Asawa T, Komiya I, Nagasawa Y, Yamada T (1991) Alloxan-induced DNA trend breaks in pancreatic islets. J Biol Chem, 266: 2112–2114.

28. Ugochukwu NH, Babady NE (2003) Antihyperglycemic effect of aqueous and ethanolic extracts of Gongronema latifolium leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Sciences, 73: 1925–1938.

29. Yanardag R, Ozsoy-Sacan O, Bolkent S, Orak H, Karabulut-Bulan O (2005) Protective effects of metformin treatment on the liver injury of streptozotocin-diabetic rats. Human Experimental Toxicol, 24: 129–135.