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ORIGINAL PAPER

Antihyperglycemic and antioxidative potential of *Matricaria chamomilla* L. in streptozotocin-induced diabetic rats

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Abstract Plants with antidiabetic activities provide important sources for the development of new drugs in the treatment of diabetes mellitus. In the present study, we investigated possible antihyperglycemic and antioxidative activities of the aerial part of the *Matricaria chamomilla* L. ethanolic extract (MCE) in streptozotocin (STZ; 70 mg/kg, i.p.)-induced diabetic rats. The following groups were assigned; sham (did not receive any substance), STZ + distilled water (control), STZ + 5 mg/kg glibenclamide, STZ + 20 mg/kg MCE, STZ + 50 mg/kg MCE, STZ + 100 mg/kg MCE. Diabetic rats were treated for 14 days by gavage. Postprandial blood glucose levels, malondialdehyde, reduced glutathione (GSH), nitrate, nitrite, ascorbic acid, retinol, β-carotene, superoxide dismutase, and catalase levels were measured, and immunohistochemical studies were performed in all of the groups. The obtained data showed that STZ resulted in oxidative stress and affected the antioxidant status. Treatment with different doses of MCE significantly reduced postprandial hyperglycemia and oxidative stress, and augmented the antioxidant system. In histological investigations, MCE treatment protected the majority of the pancreatic islet cells, with respect to the control group. As a result, MCE exhibited significant antihyperglycemic effect and protected β-cells in STZ-diabetic rats, in a dose-dependent manner, and diminished the hyperglycemia-related oxidative stress.

Keywords Streptozotocin · Diabetes · Oxidative stress · Antioxidant · Pancreas · Immunohistochemistry

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Introduction

Diabetes mellitus (DM) is a chronic and major endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a growing health problem in most countries and its incidence is considered to be high (4–5%) all over the world. Chronic hyperglycemia causes complications linked to diabetes, such as heart disease, retinopathy, kidney disease, and neuropathy. It is also a common cause of chronic morbidity and disability among the working population in the world. Several drugs, such as sulfonylureas, metaformin, and α-glucosidase inhibitors, are used presently to reduce the hyperglycemia. In spite of the use of many hypoglycemic agents, diabetes and its linked complications are still an important medical problem. All of these drugs also have limited efficacy and certain adverse effects, such as causing hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea [1]. The formation of reactive oxygen species (ROS) is involved in the etiology and pathogenesis of diabetes and

the development of diabetic complications [2]. Prolonged exposure to hyperglycemia causes oxidative stress and reduces capacities of the endogenous antioxidant defense system via the production of several reducing sugars (through glycolysis and the polyol pathway) [3]. These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation reaction), and increase the production of ROS [4]. On the other hand, it has been stated that the generation of ROS contributes to streptozotocin (STZ)-induced destruction of pancreatic β-cells [5].

Chamomile is one of the most widely used and well-documented medicinal plants in the world. The use of chamomile as a medicinal plant dates back to ancient Greece and Rome. *Matricaria chamomilla* L., locally known as "Papatya," is widely distributed throughout all regions of Turkey, and is natively found on the road-side and unused fields. Its infusion form (1%) or powder (1–2 g/day) are used internally as a diuretic, sedative, carminative, and secretogog for bile, and are used externally to treat skin wounds, mouth sores, and hemorrhoids in Turkish traditional medicine. The main active constituents of *M. chamomilla* L., grown in Turkey, are bisabolol, bisabolol-oxide, bisabolonoxide, and chamazulene. It contains 0.75% of a volatile oil [6].

Diabetes mellitus is known from ancient times onwards, and some medicinal plants are used to control diabetes in traditional medicine [7, 8]. Plants with antidiabetic activities provide important sources for the development of new drugs in the treatment of DM. In recent years, considerable focus has been given to an intensive search for a novel type of antihyperglycemic agent from numerous plant materials. In diabetes phytotherapy, the effects of *M. chamomilla* L. have never been demonstrated experimentally in either clinical or experimental diabetes. Thus, in the present study, we investigated the possible antihyperglycemic and antioxidative activities of ethanolic extract obtained from the aerial part of *M. chamomilla* L. (MCE) in STZ-induced diabetic rats. In addition, the effect of MCE is compared to glibenclamide, which was used as a reference hypoglycemic drug.

Materials and methods

Plant material

The aerial parts of *M. chamomilla* L. were collected in May 2005 from Afyonkarahisar (altitude: 1,020 m). The plant was identified by the Department of Botany of the Science and Arts Faculty, Kocatepe University, Afyonkarahisar, Turkey (Herbarium number: Kala1397). A voucher specimen (B3A) has been kept in our laboratory for future reference.

Extraction and preparation of the test samples

Air-dried *M. chamomilla* L. was pulverized with a blender. One hundred grams of this plant material was extracted on a Soxhlet apparatus using 1 l of hydroalcoholic solvent containing 37% ethanol and 63% distilled water. Finally, the solvent was recovered and the extract was lyophilized, weighed (yield: 17.7%), and stored at 4°C, and was used to treat the animals as needed. The extract (MCE) was further diluted with distilled water to obtain different doses.

Chemicals

Streptozotocin was purchased from Fluka (Germany) and superoxide dismutase (SOD) commercial kit was purchased from Randox Laboratories Ltd. (UK). Hydrogen peroxide, glutathione (GSH), thiobarbituric acid, phosphate buffer, butylated hydroxytoluene, trichloroacetic acid, EDTA, [5,5-dithiobis-(2-nitrobenzoic acid)], disodium hydrogen phosphate, phenylenediamine, sodium azide, 2,4-dinitrophenylhydrazine, ethanol, hexane, sodium nitrite, sodium nitrate, sulfanilamide, N-(1-Naphthyl) ethylenediamine dihydrochloride, and vanadium (III) chloride were purchased from Sigma (Germany). All other chemicals and reagents used in this study were of analytical grade. Glibenclamide (Gliben, Nobel İlaç A.Ş., Turkey) was purchased from a local drug store.

Animals

Fifty-four male Wistar albino rats with a weight of 190–275 g were used for the experiment. The rats were fed with standard laboratory chow and water before the experiment. They were divided into six equal groups ($n = 9$) and housed in cages. The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and approval was received from our institutional Animal Ethics Committee.

Induction of diabetes

After overnight fasting (deprived of food for 16 h but allowed free access to water), diabetes was induced in the rats by intraperitoneal injection of a freshly prepared solution of STZ (70 mg/kg, b.w.) in 0.1 M citrate buffer, pH 4.5. Control rats were injected with citrate buffer alone. After 6 days for the development of diabetes, the rats with moderate diabetes having hyperglycemia (blood glucose

range of above 180 mg/dl) were considered as diabetic rats and were used for further experiments. The treatment was started on the seventh day after STZ injection and this was considered as the first day of treatment. The treatment by gavage was continued for 14 days. The animals were divided into six equal groups. Sham rats (group 1) did not receive any substance (no diabetic). Control rats (group 2) were given vehicle (distilled water) only, while group 3 received glibenclamide suspended in distilled water orally at a dose of 5 mg/kg. Groups 4–6 received MCE suspended in distilled water orally at doses of 20, 50, and 100 mg/kg, respectively. Postprandial blood samples were collected from the tail vein and measured with a glucometer (Medi-Sense, Optium Xceed, Abbott, UK) at 0, 7, and 14 days of treatment. At the end of the experiment, all animals were sacrificed under ether anesthesia, the blood samples were taken by cardiac puncture, and the pancreata were removed for the immunohistochemical analysis.

For the assessment of hypoglycemic activity in normal healthy rats, the overnight fasted rats received 100 mg/kg MCE by gavage. Fasting blood glucose levels were measured as described above, before and after 2 h from MCE administration.

Biochemical analysis

Blood samples for the biochemical analysis were collected by cardiac puncture in heparinized and normal tubes. Whole blood was collected into heparinized tubes and whole blood malondialdehyde (MDA) and reduced GSH levels were studied on the same day of admission. Blood was also collected into a polystyrene microtube and, after clotting, this was centrifuged at 4,000 rpm for 7 min and the serum was removed using EDTA-washed Pasteur pipettes. Heparinized red blood cells were washed three times with phosphate-buffered saline, pH 7.4. The serum and erythrocyte were stored in polystyrene plastic tubes at −70°C until the time of analysis.

Whole blood MDA (as an important indicator of lipid peroxidation) levels were measured according to the method of Jain et al. [9]. The principle of the method was based on the spectrophotometric measurement of the color that occurred during the reaction of thiobarbituric acid with MDA. The concentration of thiobarbituric acid reactive substances was calculated by the absorbance coefficient of MDA–thiobarbituric acid complex and is expressed in nmol/ml. Estimation of the GSH was measured by the method of Beutler et al. [10], by a spectrophotometric method. After lysing whole blood and the removal of precipitate, disodium hydrogen phosphate and DTNB solution were added and the color formed was read at 412 nm. The results are expressed in mg/dl. The concentrations of nitric oxide (nitrate and nitrite) were detected by the methods of Miranda et al. [11]. Nitrite and nitrate calibration standards were prepared by diluting sodium nitrite and sodium nitrate in pure water. After loading the plate with samples (100 µl), the addition of vanadium (III) chloride (100 µl) to each well was rapidly followed by the addition of the Griess reagents, sulfanilamide (50 µl) and N-(1-naphthyl) ethylenediamine dihydrochloride (50 µl). The Griess solutions may also be premixed immediately prior to application to the plate. Nitrite mixed with Griess reagents forms a chromophore from the diazotization of sulfanilamide by acidic nitrite, followed by coupling with bicyclic amines, such as N-(1-naphthyl) ethylenediamine. Blank sample values were obtained by substituting a diluting medium for Griess reagent. Nitrite was measured in a similar manner, except that samples and nitrite standards were only exposed to Griess reagents. The absorbance at 540 nm was read to assess the total plasma level of nitrite and nitrate in all samples. Serum vitamin C (ascorbic acid) level was determined after derivatization with 2,4-dinitrophenylhydrazine [12]. The levels of β-carotene at 425 nm and vitamin A (retinol) at 325 nm were detected after the reaction of serum:ethanol:hexane at the ratio of 1:1:3, respectively [13]. Catalase (CAT) activity was measured according to the method of Aebi [14]. The principle of the assay is based on the determination of the rate constant (k s^{-1}) of hydrogen peroxide decomposition by the CAT enzyme. The rate constant was calculated from following formula: $k = (2.3/\Delta t)(a/b)\ \log(A_1/A_2)$. In this formula, A_1 and A_2 are the absorbance values of hydrogen peroxide at the t_1 (0th s) and t_2 (15th s) times, a is the dilution factor, and b is the hemoglobin content of erythrocytes [14]. Erythrocyte SOD activities were studied on hemolysates by using commercial kits (Randox) [15].

Pancreas immunohistochemistry

The pancreas was fixed in 10% buffered formalin and was routinely processed for embedding in paraffin. Tissue blocks were cut into 5-µm-thick sections. Immunohistochemistry was performed by the streptavidin-biotin-peroxidase complex (S-ABC) (streptavidin-biotinylated horseradish peroxidase; DakoCytomation Denmark A/S). The endogenous peroxidase and non-specific binding sites for antibodies were suppressed by treating sections with 0.3% hydrogen peroxide (2 ml of H_2O_2 in 18 ml of methanol) for 20 min and 5% normal bovine serum (1:5 diluted Tris) for 20 min at room temperature, respectively. The sections were incubated with polyclonal guinea-pig anti-insulin (1:150 diluted, A 0564; Dako, Denmark) and biotinylated link universal (K 0690, DAKO LSAB Kits),

and streptavidin-HRP (K 0690 DAKO LSAB Kits) for 30 min, respectively. Peroxidase was detected with a diaminobenzidine substrate kit (DAB kit; Sigma, 1 DAB tablets + 20 ml Tris + 20 µl H_2O_2). Finally, slides were counter-stained with Mayer's hematoxylin, dehydrated, and mounted.

For morphometry, ten Langerhans islets from each rat (90 islets for each group) were chosen randomly. The intensity of staining with antiinsulin antibodies was scored semiquantitatively as A = weak, individualized cell reactivity in ≤25% of islets; B = mild to moderate insulin reactivity in ≥25–≤50% of islets; C = moderate insulin reactivity in ≤75% of islets; D = strong reactivity in ≥75–100% of islets. The average staining intensity was calculated as $[(A \times 1) + (B \times 2) + (C \times 3) + (D \times 4)]/(A + B + C + D)$ and is reported as follows: + = 0.00–1.00; ++ = 1.01–2.00; +++ = 2.01–3.00; ++++ = 3.01–4.00. In addition, the numbers of insulin-positive cells were calculated in the 90 islets from each experimental group. The numbers were then averaged for statistical analysis and are presented as percentages of the total cells counted.

Statistical analysis

All values were expressed as mean ± SD. Statistical analysis of the data was performed using a one-way analysis of variance (ANOVA) and Tukey's posttest. A value of $P < 0.05$ was considered to be statistically significant.

Results

Table 1 shows the body weight changes in the normal and experimental animals in each group. The mean body weight of the diabetic rats was decreased as compared to sham rats (there is no significant difference between days for all groups). The body weight loss of diabetic rats treated with MCE was lower as compared to non-treated diabetic rats.

Biochemical studies

Table 2 shows the blood glucose level changes in sham and experimental animals in each group. The blood glucose levels were significantly increased in diabetic animals as compared to sham animals. However, the high levels of blood glucose decreased in diabetic rats treated with MCE and diabetic rats treated with glibenclamide. The glucose lowering effect of MCE was in a dose-dependent manner. MCE showed a maximum effect at a dose of 100 mg/kg and this effect was higher than that of glibenclamide. In normal healthy rats treated with 100 mg/kg MCE alone, there was no effect on fasting blood glucose levels (results not presented).

Figure 1 shows the levels of MDA in the whole blood of normal and experimental animals in each group. The MDA levels were greatly increased in STZ-induced diabetic rats as compared to normal rats. The treatment of STZ-induced diabetic rats with MCE or glibenclamide for 14 days resulted in a marked decrease in whole-blood MDA. The levels of MDA in diabetic rats treated with MCE were close to those in diabetic rats treated with glibenclamide.

Table 3 shows the levels of nonenzymatic antioxidants ascorbic acid, β-carotene, and retinol in the serum or GSH in the whole blood of normal and experimental animals in each group. There was no significant difference between groups of ascorbic acid levels. The treatment of STZ-induced diabetic rats with MCE caused an increase in the β-carotene levels, but only the 100-mg/kg MCE effect was significant. Retinol level in non-treated diabetic animals was lower as compared to normal animals. The treatment of STZ-induced diabetic rats with MCE (20 and 100 mg/kg effects were significant) or glibenclamide resulted in an increase in the serum retinol level. The GSH level decreased in the whole blood of untreated diabetic rats as compared to normal animals, whereas GSH levels were significantly high in MCE- or glibenclamide-treated rats as compared to normal or non-treated diabetic rats.

Table 1 Body weight changes in normal and experimental animals in each group at the initial, seventh, and 14th days after treatment. There is no significant difference between days

Groups	n	Body weight (g, mean ± SD)		
		Day 0	Day 7	Day 14
Sham	9	261.23 ± 9.3	269.22 ± 9.5	271.01 ± 15.3
Control (STZ)	9	221.56 ± 14.4	219.34 ± 14.9	209.89 ± 24.2
STZ + 5 mg/kg GLB	9	222.33 ± 16.5	223.45 ± 16.9	214.11 ± 21.5
STZ + 20 mg/kg MCE	9	215.78 ± 13.8	217.23 ± 20.5	209.44 ± 19.6
STZ + 50 mg/kg MCE	9	233.89 ± 26.1	236.11 ± 23.7	224.89 ± 23.6
STZ + 100 mg/kg MCE	9	206.67 ± 22.6	209.21 ± 33.4	203.45 ± 34.5

STZ = 70 mg/kg streptozotocin; GLB = glibenclamide; MCE = *Matricaria chamomilla* L. extract

Table 2 Changes in postprandial blood glucose level at the initial, seventh, and 14th days after treatment

Groups	*n*	Blood glucose level (mean ± SD, mg/dl)		
		Day 0	Day 7	Day 14
Sham	9	110.02 ± 8.6	113.22 ± 10.3	112.67 ± 13.6
Control (STZ)	9	186.78 ± 21.5[a]	218.89 ± 81.4	223.22 ± 32.3
STZ + 5 mg/kg GLB	9	194.33 ± 19.3[a]	176.01 ± 21.4	180.01 ± 27.2[d]
STZ + 20 mg/kg MCE	9	198.56 ± 12.2[a]	184.22 ± 18.5	187.33 ± 34.7
STZ + 50 mg/kg MCE	9	190.11 ± 27.6[a]	138.89 ± 50.5[b]	160.78 ± 33.7[d]
STZ + 100 mg/kg MCE	9	192.78 ± 16.1[a]	119.44 ± 12.1[c]	147.34 ± 17.1[d]

STZ = 70 mg/kg streptozotocin; GLB = glibenclamide; MCE = *Matricaria chamomilla* L. extract

[a] $P < 0.001$ vs. sham; [b]$P < 0.01$ vs. control; [c]$P < 0.001$ vs. control; [d]$P < 0.05$ vs. control; by ANOVA and Tukey's posttest

Table 3 Levels (mean ± SD) of nonenzymatic antioxidants ascorbic acid, β-carotene, and retinol in the serum or GSH in the whole blood of normal and experimental animals in each group

Groups	*n*	GSH (mg/dl)	Ascorbic acid (mg/dl)	β-carotene (μg/dl)	Retinol (μg/dl)
Sham	9	27.10 ± 3.9	2.48 ± 0.6	20.93 ± 5.3	96.08 ± 7.6
Control (STZ)	9	23.46 ± 3.3	2.24 ± 0.3	19.72 ± 4.4	88.06 ± 5.7
STZ + 5 mg/kg GLB	9	29.54 ± 5.7[a]	2.30 ± 0.6	18.65 ± 4.1	97.34 ± 8.7
STZ + 20 mg/kg MCE	9	30.02 ± 4.6[a]	2.57 ± 0.5	22.78 ± 5.2	98.41 ± 8.2[a]
STZ + 50 mg/kg MCE	9	30.84 ± 4.3[b]	2.34 ± 0.3	22.60 ± 5.6	95.09 ± 8.5
STZ + 100 mg/kg MCE	9	30.91 ± 3.1[b]	2.38 ± 0.5	27.61 ± 4.9[a]	99.26 ± 5.5[a]

STZ = 70 mg/kg streptozotocin; GLB = glibenclamide; MCE = *Matricaria chamomilla* L. extract

[a] $P < 0.05$ vs. control; [b]$P < 0.01$ vs. control; by ANOVA and Tukey's posttest

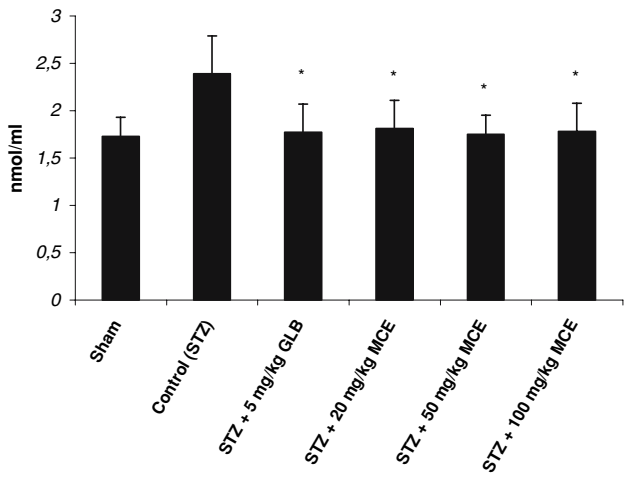

Fig. 1 The levels of malondialdehyde in the whole blood of normal (sham) and experimental animals (STZ = streptozotocin; MCE = *Matricaria chamomilla* L. ethanolic extract; GLB = glibenclamide). Each value is the mean ± SD of nine rats in each group. The *asterisks* indicate $P < 0.01$ vs. control, by ANOVA and Tukey's posttest

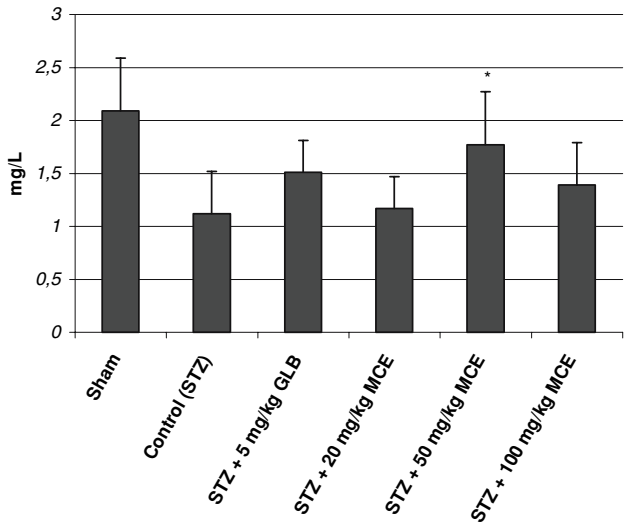

Fig. 2 The levels of nitrite in the plasma of normal (sham) and experimental animals (STZ = streptozotocin; MCE = *Matricaria chamomilla* L. ethanolic extract; GLB = glibenclamide). Each value is the mean ± SD of nine rats in each group. The *asterisk* indicates $P < 0.05$ vs. control, by ANOVA and Tukey's posttest

Figures 2 and 3 show the plasma nitrite and nitrate level changes in normal and experimental animals in each group. The plasma nitrite and nitrate levels were significantly decreased in diabetic animals as compared to normal animals. Treatment of diabetic rats with MCE (50-mg/kg effect was significant) or glibenclamide resulted in an increase in the plasma nitrite and nitrate levels. Figures 4 and 5 show the activities of enzymatic antioxidants (SOD and CAT) in

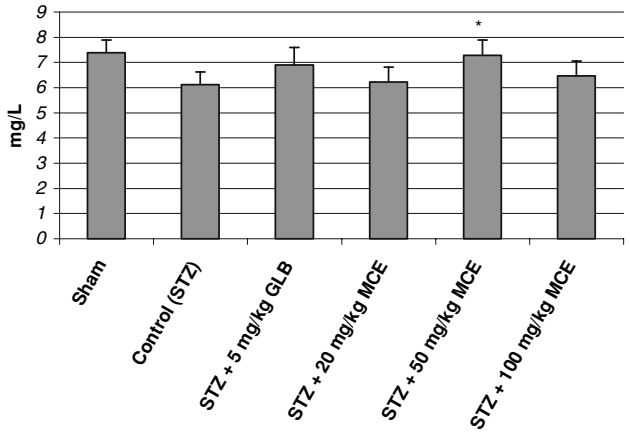

Fig. 3 The levels of nitrate in the plasma of normal (sham) and experimental animals (STZ = streptozotocin; MCE = *Matricaria chamomilla* L. ethanolic extract; GLB = glibenclamide). Each value is the mean ± SD of nine rats in each group. The *asterisk* indicates $P < 0.01$ vs. control, by ANOVA and Tukey's posttest

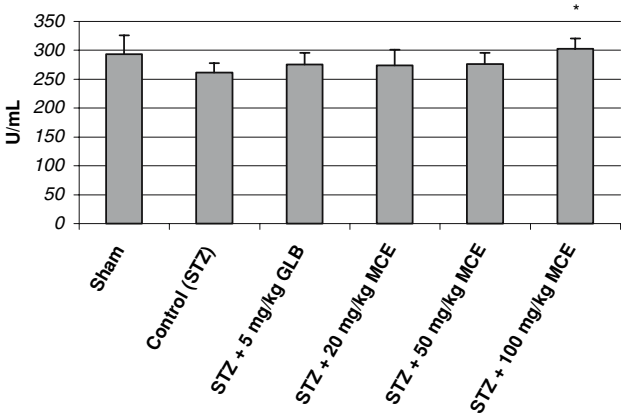

Fig. 4 The activity of superoxide dismutase in the serum of normal (sham) and experimental animals (STZ = streptozotocin; MCE = *Matricaria chamomilla* L. ethanolic extract; GLB = glibenclamide). Each value is the mean ± SD of nine rats in each group. The *asterisk* indicates $P < 0.05$ vs. control, by ANOVA and Tukey's posttest

the serum of normal and experimental animals in each group. SOD and CAT activities were decreased in STZ-induced diabetic rats as compared to normal rats. The treatment of STZ-induced diabetic rats with MCE or glibenclamide caused an increase in the SOD activity, but the 100-mg/kg MCE effect was significant with respect to non-treated diabetic rats. Moreover, all doses of MCE, but not glibenclamide, significantly increased the CAT activity of diabetic animals in each group.

Immunohistochemical study

The histology of the pancreatic islet cells was normal in the sham group. In this group, strong insulin antigen positivity was observed in the β-cells located in the center of islets, whereas insulin-negative other cells were observed in the peripheral regions of pancreatic islets. Langerhans islets showed normal histoarchitecture (Fig. 6a). There was weak insulin-immunpositivity in a few β-cells in the Langerhans islets of STZ-treated diabetic rats. In diabetic rats, the most clear findings were determined in the histologic sections of Langerhans islets, which decreased the immunopositive staining of cells for insulin and abundantly karyopyknotic cells and cytoplasmic vacuolar cells (Fig. 6b). This group showed a lack of insulin response and increased blood glucose levels (223.22 mg/dl). The insulin-immunopositive cell density was reduced by approximately 60% compared with the sham group (22.52 and 80.30%, respectively). On the other hand, these abnormal histological signs were dramatically and dose-dependently decreased in the MCE 20, 50, and 100 mg/kg dosing groups compared to that of non-treated diabetic animals (Fig. 6d–f, respectively). Moderate insulin-immunpositivity, light vacuolar

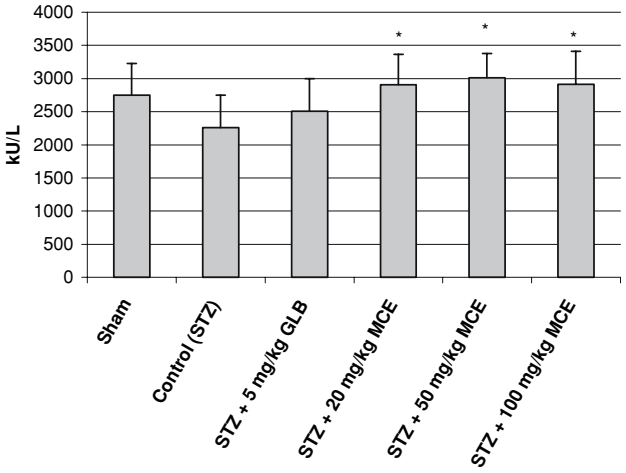

Fig. 5 The activity of catalase in the serum of normal (sham) and experimental animals (STZ = streptozotocin; MCE = *Matricaria chamomilla* L. ethanolic extract; GLB glibenclamide). Each value is the mean ± SD of nine rats in each group. The *asterisks* indicate $P < 0.05$ vs. control, by ANOVA and Tukey's posttest

degeneration and degranulation, and a few pyknotic cells were observed in the majority of β-cells of the islets in the diabetic MCE 50 and 100 mg/kg treated group. In the MCE 20, 50, and 100 mg/kg dosing groups, the blood glucose levels were decreased compared with the STZ-only group (STZ + MCE 20 mg/kg = 187.33 mg/dl, STZ + MCE 50 mg/kg = 160.78 mg/dl, STZ + MCE 100 mg/kg = 147.34 mg/dl, and STZ = 223.22 mg/dl). On the other hand, histopathological changes in the glibenclamide 5 mg/kg dosing group were very close to the MCE 100 mg/kg dosing group (Fig. 6c). As shown in Table 4, the percentage of insulin-immunopositive β-cells was calculated according to the groups. MCE treatment protected the intensity of the immunohistochemical staining of insulin in

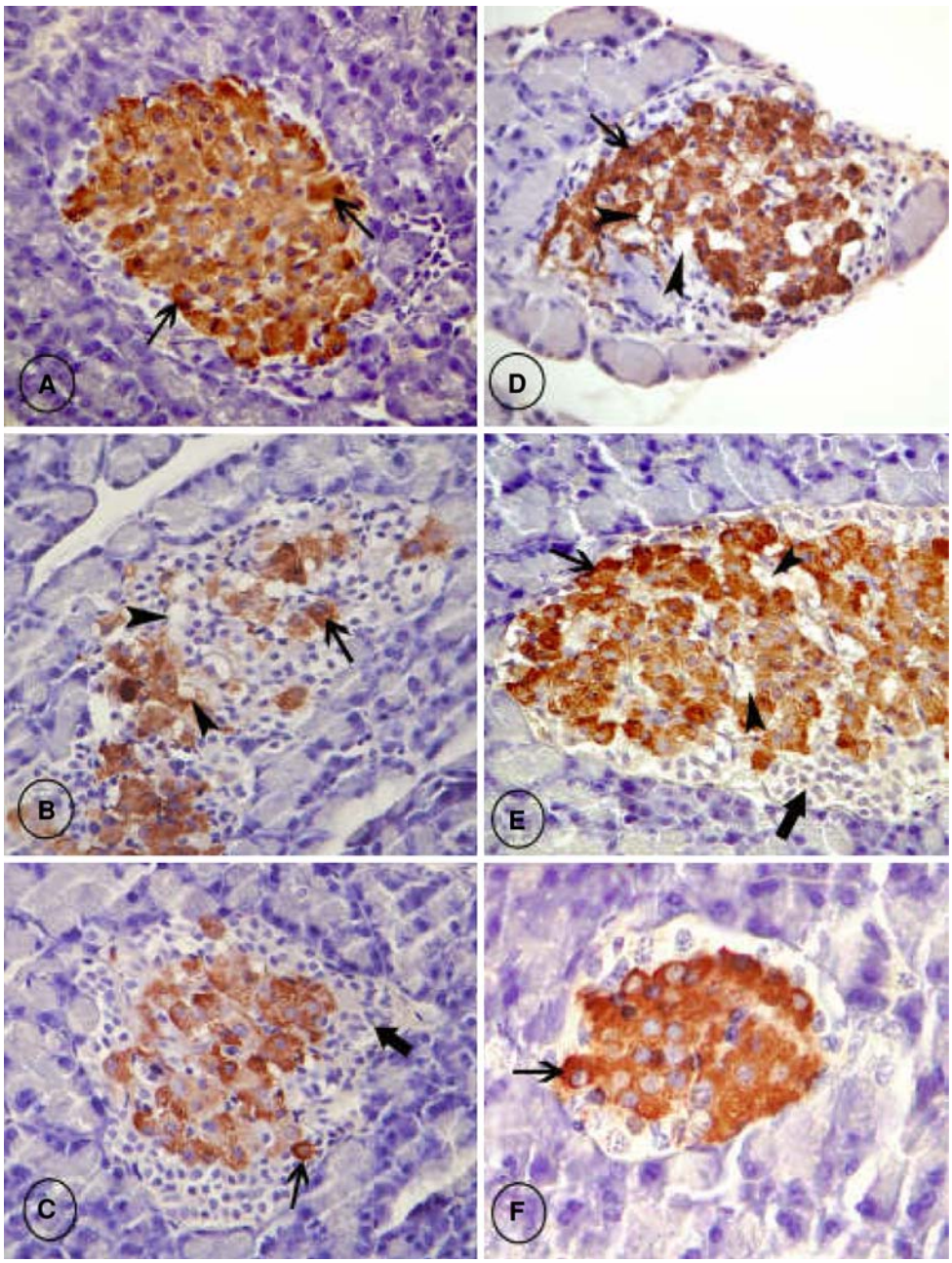

Fig. 6a–f Insulin-producing cells in the pancreatic islets in the sham (**a**), control (**b**), glibenclamide 5 mg/kg (**c**), MCE 20 (**d**), 50 (**e**), and 100 (**f**) mg/kg dosing groups. The *thin arrows* indicate insulin-immunopositive cells; the *bold arrows* indicate insulin-immunonegative cells; the *arrow heads* indicate degenerative and vacuoles cells

Langerhans islets significantly compared to the STZ–treated diabetic animals. There were no significant differences found in the intensity of insulin-immunopositive β-cells in the 100 mg/kg MCE treated group when compared to the STZ + 5 mg/kg GLB treated group.

Discussion

Diabetes mellitus (DM) is the most common endocrine disorder and is an important health problem worldwide that is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Strict control of the blood glucose level is considered to be essential in order to delay and/or prevent the development of diabetic complications. Several studies have focused on the etiology and pathogenesis of DM and its complications, in which lipid peroxidation and ROS have played a role [16, 17]. An imbalance between ROS generation and the reduced activity of antioxidant defenses or both of these phenomena lead to oxidative stress. Hyperglycemia is a cause of the oxidative stress in diabetic patients and reduces the capacity of the endogenous

Table 4 Semi-quantitative analysis of the immunohistochemical staining of insulin in β-cells in pancreatic islets of the experimental groups

Groups	*n*	Insulin-immunopositive cells (%)	Average of islets (density)	Islet staining (intensity)
Sham	9	80.30 ± 10.49	3.22	++++
Control (STZ)	9	22.52 ± 5.62	1.05	++
STZ + 5 mg/kg GLB	9	48.08 ± 5.78[a]	2.85	+++
STZ + 20 mg/kg MCE	9	25.35 ± 6.56	2.26	+++
STZ + 50 mg/kg MCE	9	37.25 ± 3.45[b]	2.48	+++
STZ + 100 mg/kg MCE	9	56.61 ± 9.17[a]	3.05	++++

Average staining intensity was calculated as $[(A \times 1) + (B \times 2) + (C \times 3) + (D \times 4)]/(A + B + C + D)$ and are reported as follows: + = 0.00–1.00; ++ = 1.01–2.00; +++ = 2.01–3.00; ++++ = 3.01–4.00. A = weak, individualized cell reactivity in ≤25% of islets; B = mild to moderate insulin reactivity in ≥25–≤50% of islets; C = moderate insulin reactivity in ≤75% of islets; D = strong reactivity in ≥75–100% of islets

STZ = 70 mg/kg streptozotocin; GLB glibenclamide; MCE = *Matricaria chamomilla* L. extract

[a] $P < 0.001$ vs. control; [b] $P < 0.01$ vs. control; by ANOVA and Tukey's posttest

antioxidant defense system via the production of several reducing sugars (through glycolysis and the polyol pathway) [3]. In our study, MCE significantly decreased hyperglycemia and lipid peroxidation, and increased enzymatic or nonenzymatic antioxidative defense systems. Therefore, it may be said that the antioxidative activities of MCE in STZ-induced diabetes, at least in part, may be related to antihyperglycemic capability.

The present study shows, for the first time, the antihyperglycemic and antioxidative properties of *M. chamomilla* L. in rats. It appears that, unlike insulin, the MCE does not have severe hypoglycemic action. When the dose of the MCE was increased from 20 to 100 mg, it resulted in a proportional decrease in the serum glucose. Further, the MCE had no hypoglycemic effect in fasted normal rats. The underlying mechanism(s) of this blood glucose lowering activity may be the stimulation of peripheral glucose utilization, especially in muscle and adipose tissue, and/or the restoration of enzyme activity which play a part in the glucose and glycogen metabolism.

Streptozotocin [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose] is a naturally produced antibiotic from *Streptomyces achromogenes* [5]. STZ-induced hyperglycemia is a widely used experimental model for screening the activity of hypoglycemic agents. In this model, hyperglycemia arises because of irreversible destruction of the β-islet cells of the pancreas by STZ, causing a reduction of insulin secretion. The generation of ROS and the subsequent increase of local oxidative stress, DNA methylation, and protein modification are suggested as the pathophysiological mechanisms of STZ-induced diabetes [5, 18]. Antioxidants were considered to be promising agents against STZ-induced diabetes due to diminishing oxidative stress by inhibiting ROS generation and lipid peroxidation [16, 19, 20].

Reactive oxygen species react with lipids and cause peroxidative changes that result in elevated lipid peroxidation. The increase in lipid peroxidation might be an indication of a decrease in enzymatic and nonenzymatic antioxidants of defense mechanisms. Free radical scavenger GSH and other nonenzymatic antioxidants play a role in the repair of free radicals exposed to biological damage. In the present study, STZ caused an increase in the lipid peroxidation (MDA level) and a decrease the nonenzymatic antioxidants (levels of β-carotene and retinol in serum or GSH in whole blood) in diabetic rats, as compared to normal rats. The decrease of nonenzymatic antioxidants levels might be due to increased utilization for scavenging free radicals. The treatment of STZ-induced diabetic rats with different doses of MCE for 14 days resulted in a marked decrease in the MDA and increase in the β-carotene, retinol, and GSH levels. The cause of the increase in the nonenzymatic antioxidants levels in treated diabetic rats might be due to decreased utilization, as lipid peroxidation is low.

Apart from the nonenzymatic antioxidants, enzymatic antioxidants such SOD and CAT play an important role in preventing the cells from being exposed to oxidative damage [21]. SOD is an enzymatic antioxidant which catalyzes the conversion of superoxide radical to hydrogen peroxide (not a free radical itself, but a reactive molecule) and molecular oxygen. Other enzymatic antioxidant CAT catalyzes the reduction of hydrogen peroxides and protects the tissues against reactive hydroxyl radicals. Decreased activities of enzymatic antioxidants such as SOD and CAT have been well-documented in STZ-induced diabetic rats [22]. The present study revealed the decreased activity of serum SOD and CAT in diabetic rats, as reported previously, which could be due to increased consumption for free radicals' detoxification. Treatment with MCE and

glibenclamide has increased the activities of serum SOD and CAT, which could be a result of decreased lipid peroxidation and/or decreased utilization.

It is well known that nitric oxide (NO) possesses both antioxidant and pro-oxidant activity. Many of the toxic actions of NO are not due directly to NO itself, but are mediated by the highly reactive oxidant compound peroxynitrite. An antioxidative property of NO has been reported by some investigators [23, 24]. Furthermore, some studies revealed the protective effects of the NO-generating compounds L-arginine and sodium nitroprusside in STZ-diabetic rats [25, 26]. NO seems to be a potential antioxidant in the present study. NO is an effective chain-breaking antioxidant in free radical-mediated lipid peroxidation, and reacts rapidly with peroxyl radicals as a sacrificial chain-terminating antioxidant. The antioxidant effect of NO on lipid peroxidation has been explained by terminating the radical chain reaction through the reaction of NO with the lipid peroxy radical (ROO) to form adducts by the below equation:

$$4NO + 2ROO + H_2O \rightarrow 2ROONO + 2NO + H_2O \rightarrow RONO_2 + RONO + 2HNO_2$$

In the present study, we found that lipid peroxidation was increased, while the plasma levels of nitrate and nitrite were decreased in untreated diabetic rats. Again, MCE treatment increased the nitrate and nitrite levels compared to untreated diabetic rats. The generation of peroxynitrite can also inhibit SOD and other antioxidant molecules and systems. The SOD level was detected to be lower in the untreated group than that of treated groups. The cause of the decrease in the untreated group may be related to the overformation of peroxynitrite from the NO. The effect of MCE on the NO pathway may be mediated by either an activation of constitutively expressed NOS or by an inhibition of NO degradation. Thus, it may be suggested that the protective effect of MCE against oxidative stress, at least in part, may be related to the restoration of NO availability.

In our study, decreased immunohistochemical staining for insulin in pancreatic islets was detected in the non-treated diabetic animals. Again, vacuolation and necrotic degeneration in the central part of the islets were observed. But these abnormal histological signs were dramatically and dose-dependently decreased in the MCE dosing groups compared to that of the non-treated diabetic group. There were more insulin-positive β-cells in the islets of the MCE-dosing diabetic rats than those of the non-treated rats. The highest dose of MCE was most effective and the appearance of its islets were similar to normal rats. MCE has significantly protected islet cells against STZ-induced destruction and this effect may be partly related to the antioxidative properties of MCE.

In conclusion, the results of this study represent that the administration of MCE showed antihyperglycemic effect, which controls the blood glucose level and, thereby, inhibits the formation of free radicals or it may scavenge the reactive oxygen metabolites through various antioxidant compounds in them. On the other hand, MCE has a favorable effect to inhibit the histopathological changes of the pancreas in STZ-induced diabetes. Therefore, *M. chamomilla* L. may provide new alternatives for the clinical management of diabetes and the consumption of *M. chamomilla* L. aerial part can prevent the complications of hyperglycemia associated with diabetes. Further studies will be needed to determine the active components in MCE and their role in controlling diabetes, and to reveal the exact underlying mechanism(s) on how the MCE can treat diabetes.

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