Molecular Biology Research Communications 2013;2(4):101-107 MBRC

Original Article Open Access

Effects of *Teucrium polium* aerial parts extracts on Malonyl-CoA decarboxylase level

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A B S T R A C T

Malonyl-CoA decarboxylase (MCD) is an enzyme involved in the decarboxylation of malonyl-CoA to acetyl-CoA. In order to explore the hypothesis that the changing plant materials' MCD activity level can serve as therapy to diabetics, the effect of *Teucrium polium* compounds was studied in a diabetic rat model. In this experimental study, two groups of rats, a control and a diabetic group, each including six rats, were used. At the end of the experiment, all rats were exterminated by ether anesthesia, their pancreases removed and dissected. Isolated rat pancreas was cultured in buffers with or without 100-500µg/l *T. polium* aerial parts extracts containing arginine and leucine. MCD and insulin levels were measured after culture at 37°C and 5% CO2, for 1, 3 and 5 days. Results showed that *T. polium* aqueous and the alcoholic extract decreased MCD activity. Present data also indicate that incubation of pancreatic tissue at a concentration of 2.8 and 16.7 mmol/L glucose stimulated insulin release. For the first time it seems that aqueous and alcoholic extracts of this plant decreased MCD activity.

Key words: Aerial parts; *Teucrium polium*; malonyl-CoA decarboxylase; MCD; Insulin

INTRODUCTION

Amongst the serious metabolic disorders, diabetes mellitus (DM) is a common disease throughout the world. For the treatment of this widespread disorder, traditional plants have been used for many years. Herbalists propose various kinds of plants against the hyperglycemia in this disease [1]. Several studies have been investigated on the hypoglycemic effects of *Teucrium polium* [2-4]. Some have shown that the administration

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pISSN 2322-181X eISSN 2345-2005

of a *T. polium* crude extract to STZ-induced diabetic rats can significantly decrease serum glucose values and enhance serum insulin levels [5-6]. It is important to emphasize that malonyl-CoA decarboxylase (MCD) is the enzyme responsible for decarboxylating malonyl-CoA to acetyl- CoA [7]. Malonyl-CoA is also produced by the enzymatic activity of acetyl CoA carboxylase (ACC), which catalyses the carboxylation of acetyl CoA to malonyl-CoA . Earlier studies have suggested that in certain circumstances, the concentration of malonyl-CoA in the liver and muscles correlates inversely with changes in MCD activity [8]. Molonyl-CoA is considered to be a key factor in energy balance [9]. In addition, malonyl-CoA is involved in the control of insulin secretion and insulin sensitivity, and because of its effects on the brain, it is a key fuel sensor for food intake [10-11].

Many investigators have reported that increases in MCD activity are observed in rat liver during starvation [12]. Recent data support the proposition that MCD can be targeted for the treatment of metabolic diseases [13]. In recent years, there has been growing interest in designing a new class of MCD inhibitors with anti-obesity and anti-diabetic activities [10]. Given the above findings, to the aim of the present study was to discover the possible effect of *T. polium* aerial parts extracts on MCD levels.

MATERIALS AND METHODS

Plant material: *T. polium* aerial parts were collected in august 2010 from the suburbs of Babol city, Iran. The plant was identified by the center for Agricultural Research and Natural Resources of Mazandaran Province, Iran. The aerial parts were separated and dried at room temperature away from direct sunlight and were then grounded into powder.

Preparation of the aqueous and alcoholic extract of *T. polium*: In order to prepare the aqueous concentrate, 100 g of dry *T. polium* aerial parts were dried at room temperature and infused and boiled in two litres of ionized water (Behdad – water bath, 80ºC) for 30 minutes with occasional stirring. The resulting mixture was filtered and non soluble parts were separated by a mesh. The aqueous solution was then passed through Whatman paper No. 2 twice and evaporated on a rotator evaporator (K-1Karwerke, GMBH S Co. KG, Germany, TYP: RVo6-ML, 010388949) at 75°C, to reduce volume to a fifth of the initial value. For the preparation of the alcoholic solution, 100 g of dry powder was dissolved in one litre of 96% ethanol and mixed by a shaker (Labtron –Ls-100) for 24 hours at room temperature. Again, the non-soluble parts were separated using a mesh and the solution was passed through Whatman paper No. 2 twice. The macerate was filtered, and ethanol was evaporated on a rotator evaporator at 60°C in vacuum conditions to reduce volume to a fifth of the initial value. Both aqueous and ethanol solutions were kept under a hood for four days to allow solvents to evaporate. Finally, the extracts were stored at -20ºC until use.

Column Separation: The extracts of *T. polium* aerial parts extracts were applied to a column to obtain sufficient concentration. Ethanol or water elution mediums were both used. Aliquots (200 µl) of the aqueous and ethanol extracts were applied 15 cm over the

column (2mm, gel). Each fraction was then separated by centrifugation at 3000*g. The supernatants were separated and washed with ethanol or water. After this, each fraction was dried and weighed. The fractions were then mixed with p-anisaldehyde and sulfuric acid reagent and heated at 95°C for optimal color development. The elution was monitored at 360-380 nm.

Animals: Male adult Wistar rats weighing between 200-250 g were fed on pellet diet and tap water for full acclimatization. Closed colony rats were brought from the animal center of Babol University, Babol, Iran, and divided into two groups; a control group (n=6), and a diabetic group (n=6). Age-matched rats were used as control animals. The rats were housed in suspended bracket cages in a climate-controlled room at 22±5 ºC with a 12L: 12D lighting cycle. Feed and water were provided ad libitum. All procedures were in accordance with the animal experimental guidelines of Babol University. Also, all protocols involving animals were approved by Babol University Animal Care and Use Committee. All experimental manipulations were carried out with the animal under ether inhalation anesthesia. The approval of the Ethics Committee of Babol University was also obtained (# 3546, and 1815).

Streptozotocin Induced diabetes: Streptozotocin (STZ; Sigma, USA) was dissolved in cold 0.9% saline just before use and injected intraperitoneally (i.p.) to the rats. Diabetes was induced by a single i.p. injection of STZ (50 mg/kg body weight) to overnight fasted rats. In addition, normal saline was injected to rats in the control group. Rats with fasting glycemia more than 250 mg/dl were used as diabetic. Blood samples for glucose measurements were taken from the tail vein about 72 h after STZ injection. Diabetes was confirmed by measuring the glucose concentration using the glucose oxidase method.

Preparation of pancreatic homogenate: For in vitro experiment, the pancreas was removed from freshly exterminated rats and stored at −80ºC. Upon completion of the experimental period, the rats in both groups were exterminated by cervical dislocation and their pancreases were removed and washed with ice-cold physiological saline solution. The pancreas was cut into small pieces and subjected to digestion for 15 min at 37ºC on a magnetic stirrer. The dissociation medium consisted of Hank's balanced salt solution (HBSS), pH 7.2 with which the digested tissue was finally washed three times.

In vitro plant extracts treatment: Freshly digested pancreatic tissue (50 mg) was transferred into plastic Petri dishes and pre-incubated in RPMI 1640 medium containing 4 mmol of leucine and arginine at pH 7.2 without glucose for 30 min. After pre-incubation, the pancreatic homogenates were washed twice with HBSS buffer and incubated in 1 ml of the RPMI 1640 buffer with 2.8 mM glucose for 60 min. Purified TP extract (concentration: 0,100,200,500 µg/l) and 1 ml of RPMI 1640 were then added to each Petri dish and incubated for 1, 3 and 5 days at 37ºC and 5% CO2. Following this, the supernatant was removed from each well and centrifuged (1200g, 10 min, 5ºC). Finally, 0.5 ml of the supernatant was taken, and insulin and malonyl-CoA decarboxylase activity levels were assayed by the ELISA method.

Biochemical assay: Glucose contents were measured spectrophotometrically (Jenway, Model 6505 and UK) using the glucose oxidase method (Pars AzmmonCo., Tehran, IRAN). The insulin level was measured following the ELISA assay by the insulin kit specific to rats, made by Mercodia Rat insulin ELISA (10-1250-01,Mercodia AB, Uppsala, Sweden) and an ELISA reader. MCD activity level was determined by the ELISA assay method using a Rat malonyl coenzyme A decarboxylase ELISA kit (CSB-E14337r, Cusabio Biotech Co., LTD).

Statistical analysis: All values have been presented as means ± standard errors. Statistical analysis was done using SPSS version 16.0. ANOVAs and unpaired two-tailed Student's t-tests were run to find significant differences between the obtained means. A *p* value less than 0.05 was considered statistically significant**.**

RESULTS

The present study showed that 100-500 µg of the *T. polium* aerial parts extracts powder/mL caused a significant ($P<0.05$) increase in insulin release during a six week period, starting from the basal level of 0.0.32 ± 0.11 µg/l at the 1st day to a peak value of 0.85 ± 0.22 µg/l at the 5th day (at 2.8 mmol/L glucose).

Statistical analysis showed that 100-500 µg of the *T. polium* aerial parts extracts powder/mL caused a significant ($P<0.05$) decrease in MCD level during a six week period, from the basal level of 31.43±1.25 pg/ml at the 1st day to a peak value of 19.54±5.36 pg/ml at the5th day (at 2.8 mmol/L glucose).

Pancreatic tissue was incubated with a dose of 100-500 µg/l *T. polium* aerial parts extracts for 1, 3 and 5 days in the presence of 2.8 and/or 16.7 mmol/l glucose. The addition of *T. polium* aerial parts extracts increased insulin secretion compared to the untreated group [0.85±0.22 vs. 0.32±0.11 µg/l, ($P< 0.05$)] at 2.8 mmol/l glucose concentration and [0.83±0.21 vs. 0.28±0.08] µg/l, ($P<0.05$) at 16.7 mmol/l glucose concentration, respectively. When rat pancreatic were challenged with 2.8 mmol/l glucose in the presence of *T. polium* aerial parts extracts, MCD was inhibited compared to the untreated group [19.54±5.36 vs. 31.43±1.25 pg/ml, ($P<0.05$)], and [20.58±5.14 vs.32.46±1.32 pg/ml($P< 0.05$)] at 16.7 mmol/l glucose concentration, respectively. According to our findings, *T. polium* aerial parts extracts caused a small increase in insulin secretion (Tables1 and 2).

Table 1. Insulin level (µg/l) at incubated pancreatic tissue with dose 100µg/l *Teucrium polium* aerial parts extracts for 1, 3 and 5 days in the presence of 2.8 mmol/l glucose.

Time/groups	Control	Diabetic	Treated with aqueous extract	Treated with ethanolic extract
1st day	0.58 ± 0.11	0.41 ± 0.09	0.32 ± 0.07	0.81 ± 0.12
3th day	0.77 ± 0.13	0.59 ± 0.10	0.36 ± 0.08	0.83 ± 0.14
5th day	0.85 ± 0.15	0.63 ± 0.12	0.37 ± 0.09	0.87 ± 0.16

Data are expressed as means ± SD, P<0.05.

Table 2. Insulin level (µg/l) at incubated pancreatic tissue with dose 100 µg/l *Teucrium polium* aerial parts extracts for 1, 3 and 5 days in the presence of 16.7 mmol/l glucose.

Time/groups	Control	Diabetic	Treated with aqueous extract	Treated with ethanolic extract
1st day	0.55 ± 0.09	0.37 ± 0.07	0.28 ± 0.04	0.92 ± 0.10
3th day	0.76 ± 0.11	0.58 ± 0.11	0.35 ± 0.06	0.95 ± 0.15
5th day	0.83 ± 0.18	0.72 ± 0.14	0.41 ± 0.08	0.98 ± 0.18

Data are expressed as means ± SD, P<0.05.

The results indicate that *T. polium* aerial parts extracts were found to decrease MCD level in normal animals and those made hyperglycemic with STZ. The effects of *T. polium* aerial parts extracts in STZ induced hyperglycemic rats are shown in the (Tables 3, 4).

Table 3. MCD level (pg/ml) at incubated pancreatic tissue with dose 100µg/l Teucrium polium aerial parts extracts for 1,3 and 5 days in the presence of 2.8 mmol/l glucose.

Time/groups	Control	Diabetic	Treated with aqueous extract	Treated with ethanolic extract
1st day	21.62 ± 2.34	28.68 ± 2.11	31.43 ± 1.89	19.06 ± 1.12
3th day	20.13 ± 1.68	27.81 ± 2.14	37.89 ± 2.11	19.38 ± 1.14
5th day	19.54 ± 1.53	26.12 ± 2.31	41.27 ± 3.06	21.16 ± 1.17

Data are expressed as means ± SD , P<0.05.

Table 4. MCD level (pg/ml) at incubated pancreatic tissue with dose 100 µg/l *Teucrium polium* aerial parts extracts for 1, 3 and 5 days in the presence of 16.7 mmol/l glucose.

Time/groups	Control	Diabetic	Treated with aqueous extract	Treated with ethanolic extract
1st day	20.46 ± 2.36	27.68 ± 2.12	32.46 ± 2.19	21.17 ± 1.15
3th day	21.36 ± 1.09	26.84 ± 2.13	31.78 ± 2.09	22.45 ± 1.16
5th day	20.58 ± 1.23	25.16 ± 2.33	30.85 ± 2.98	23.78 ± 1.18

Data are expressed as means ± SD, P<0.05.

DISCUSSION

Our results showed that in a dose range equivalent to 100-500 µg of the plant powder/ml, *T. polium* aerial parts extracts were capable of enhancing insulin release (at 2.8 mmol/l glucose) compared to the untreated pancreatic. However, the insulinotropic effect of *T. polium* aerial parts extracts was more evident at higher glucose (at 16.7 mmol/l) concentrations.

Generally, in situations where the MCD level is elevated, malonyl-CoA content is low, resulting in elevated rates of fatty acid oxidation. The increase in fatty acid oxidation that occurs in hyperglycemic rats is associated with an increase in MCD activity. Our data suggest that decreased MCD activity may contribute significantly to the low fatty acid oxidation rates in the STZ-induced diabetic rats due to the fact that MCD is involved in the decarboxylation of malonyl-CoA to acetyl-CoA. Therefore, a decrease in MCD level may decrease fatty acid oxidation as well. The level of MCD with increasing content of glucose

above the physiological range decreases, it indicates that MCD can play a role in the release of insulin, and can act as a fuel sensor.The probable effects of hyperglycemia on MCD activity are not clear, thus the mechanism for this phenomenon cannot be explained at this stage.

In the present study it has been demonstrated that MCD inhibition improves pancreatic function during diabetes. MCD is a major regulator of pancreatic fatty acid oxidation, secondary only to modifying intracellular malonyl-CoA levels.

Results suggest that the pharmacological inhibition of MCD may be a viable approach to the treatment of clinical pathologies associated with hyperglycemia. The STZ diabetic rats treated with alcoholic and aqueous extracts of *T. polium* aerial parts showed a significant rise in insulin levels and a decline in MCD level compared to the untreated STZ-induced rats. We suggest the following reasons for the reduction of glucose after treatment with *T. polium* aerial parts extracts; for one thing, the inhibition of MCD might accelerate glucose oxidation. Another possible explanation is that MCD inhibition might improve functional recovery in hyperglycemic rats. Finally, it is possible that the treatment with aqueous extracts lowers insulin levels due to its chemical consistency. At any rate, the mechanism of action remains unclear and further study is needed for it to be clarified.

In conclusion, our results have shown that *T. polium* aerial parts extracts possess a hypoglycemic effect in STZ -diabetic rats. Also, for the first time we show that aqueous and alcoholic extracts of this plant decrease MCD activity levels. Referring to our results, it would be reasonable to propose that the reduction of glucose properties of *T. polium* aerial parts extracts can be attributed to MCD inhibition, suggesting that MCD can be targeted for the treatment of diabetes.

Acknowledgements

We express our gratitude to the staff of Departments of Biochemistry and Anatomical Sciences, Babol University School of Medicine for their assistance in blood collection and pancreas tissue samples. This investigation was a collaborative work of the Cellular and Molecular Biology Research Center and the Faculty of Medicine. Financial aid has been provided by the Research Council of Babol University of Medical Sciences (grants # 8930336, 1390/2/21 and # 8929121) Also, we would like to thank Mr. Shikhzadeh for his excellent technical assistance.

Conflict of Interest: The authors declare no conflicts of interest.

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