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ORIGINAL ARTICLE

The ameliorative effect of *Cetraria islandica* against diabetes-induced genetic and oxidative damage in human blood

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Abstract

Context: The aqueous extracts of *Cetraria islandica* (L.) Ach. (Parmeliaceae) is traditionally used in many countries against a number of conditions, including inflammatory conditions.
Objective: The present study aimed to assess, for the first time, the effectiveness of *C. islandica* in cultured primary blood cells of Type 1 diabetes subjects.
Materials and methods: Diabetic and control blood samples were treated with or without aqueous lichen extract (5 and 10 $\mu g\, mL^{-1}$) for 48 h. The activity of antioxidant enzymes in erythrocytes and also malondialdehyde levels in plasma were determined to evaluate the oxidative status. DNA damages were analyzed by SCE, MN and comet assays in cultured human lymphocytes. Additionally, proliferation index (PI) was evaluated in peripheral blood lymphocytes.
Results: There were significant increases in observed total DNA damage (comet assay) (240.2%) and SCE (168.8%), but not in MN frequencies of cultures with diabetes as compared ($p > 0.05$) to controls. Whereas, the significant reductions of total DNA damage (69.2 and 65.3%) and SCE frequencies (17.7 and 12.3%) were determined when the 5 and 10 $mg\, mL^{-1}$ lichen extract was added to the cell culture medium, respectively. However, lichen extract did not completely inhibit the induction of SCEs in lymphocytes of patients with diabetes. *C. islandica* extract was also useful on PI rates.
Discussion: In conclusion, the antioxidant role of *C. islandica* in alleviating diabetes-induced genomic instability and for increasing cell viability was firstly indicated in the present study.

Keywords

Diabetes mellitus, genotoxicity, human blood cultures, lichen, oxidative stress

History

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Introduction

Lichens are ecologically obligate symbiotic associations between a fungus (mycobiont) and at least one photosynthetic organism (photobionts; an alga, a cyanobacterium or both) (Widmer et al., 2012). We documented that they are effective in the treatment of tuberculosis (Vartia, 1973), hemorrhoids and dysentery (Dülger et al., 1998) and induce apoptosis in colon (Bezivin et al., 2003; Ren et al., 2009) and prostate cancers (Mitrović et al., 2011; Russo et al., 2006). The lichens also have antioxidant, antimicrobial and anticancer properties (Cansaran, 2010; Cansaran et al., 2007; Halici et al. 2005; Odabasoglu et al., 2006; Kosanic & Rankovic, 2011; Zambare & Christopher, 2012). Unique lichen flora has attracted many researchers on a systematic basis (Aslan et al., 2002). It is pointed out that lichens may be easily accessible sources of natural drugs that could be used as a possible food supplement or in the pharmaceutical industry (Archer et al., 2008).

Type 1 diabetes (T1D) is a chronic condition with a rising incidence worldwide in developed as well as in developing countries (Malerbi et al., 2012). Because of the early age of onset and longer diabetes duration, children and adolescents are at risk for developing diabetes-related complications at a younger age. As these youth age, this profoundly affects their productivity, quality of life, and life expectancy and increases health care costs (Imperatore et al., 2012). Over the past two decades our increased understanding of the pathogenesis of this disease has led to the development of new treatments. Oxidative stress has been implicated in the T1D pathogenesis (Bhatti et al., 2011; Lao-ong et al., 2012). In recent years, there has been a growing interest in the antioxidant defense system as a regulator of disease development. This opens up an important strategy for therapy of diabetes and may provide a promising avenue for future approaches to lichens. In response to pharmacological activation or oxidative stress, we studied well-known lichen species *Cetraria islandica* Ach. (1830) (Parmeliaceae) in relation to the survival of the blood cells in subjects with T1D. In many countries, *C. islandica* is used medicinally, e.g., for colds, bronchitis, and asthma (Safonova et al., 1999; Senchilo & Senchilo, 2004) and is studied for antioxidant properties with *in vitro* conditions (Kotan et al., 2011; Odabasoglu et al., 2006). The medicine is usually taken in the form of a tea (decoction or infusion) of

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the dry lichen. *C. islandica* extract demonstrates hepatoprotective and also immune-stimulating effects in blood tissue (Cernescu et al., 2011a; Freysdottir et al., 2008). Our study investigated the efficacy of *C. islandica* against diabetes-induced DNA damage with *in vitro* conditions. Firstly, some oxidative parameters including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) which are used to monitor the development and extent of damage due to oxidative stress in human blood were investigated. Secondly, genotoxicity using single cell gel electrophoresis (SCGE or comet), sister-chromatid exchange (SCE), micronucleus (MN) assays, which are rapid and sensitive methods for measuring the genetic damage, were evaluated. In addition, the proliferation index (PI) rate was assessed to provide information on cell viability. Ultimately, the data may be used to devise strategies to reduce the risk of T1D in humans. To our knowledge, these results provide for the first time cellular information of *C. islandica* on blood of humans with T1D.

Materials and methods

Lichen extract and dose adjustment

C. islandica was collected from the Giresun, Erzurum and Artvin province in Turkey, during summer of 2009. The samples were identified using various flora books and papers (Aslan, 2000; Aslan et al., 2002). The identified sample was air-dried and stored in the herbarium of Kazım Karabekir Education Faculty. For water extraction of lichen, 20 g sample was mixed with 400 mL distillated and boiling water using magnetic stirrer for 15 min. Then the extract was filtered over Whatmann No. 1 paper.

Experimental design and protocol

The study described effects of *C. islandica* in blood cultures of patients with T1D. Thus, this study is divided into two sections. Section 1: The assessment of antioxidant role of *C. islandica* on T1D-induced oxidative stress and Section 2: Study of effectiveness of *C. islandica* on T1D-induced DNA damage.

Blood samples were obtained by vein puncture from 25 children (age 10–18 years) diagnosed with T1D (disease duration <18 months) and also age and sex matched control subjects. Human peripheral blood lymphocyte cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975). The heparinized blood (0.5 mL) was cultured in 5 mL of culture medium (Chromosome Medium B, Biochroms, Berlin) with 5 mg mL^{-1} of phytohemagglutinin (Biochrome®, Berlin, Germany). *C. islandica* extract (5 and 10 µg mL^{-1}) was added to the cultures just before incubation for biochemical and cytogenetic analysis. The experiments were performed on different groups as follows: T1D alone, *C. islandica* alone and T1D plus *C. islandica*. The dosages of the lichen extract were determined by preliminary dose-response work in our laboratory and also the literature data (Kotan et al., 2011). The study was performed in accordance with the Declaration of Helsinki and with the approval of the local ethics committee. In the patients with T1D, after supplementation of *C. islandica*, the blood was incubated for 2 h at 37 °C to adjust body conditions, except for testing comet, SCE, MN and PI (see below). Each individual whole blood culture without disease or lichen extract was studied as a control group ($n = 8$).

Erythrocytes

Erythrocytes were obtained from heparinized blood samples by centrifugation (2500 *g*, for 20 min) at 4 °C. The red cells were then washed three times with 5 volumes of phosphate buffered saline (PBS; 150 mmol L^{-1} NaCl, 1.9 mmol L^{-1} NaH_2PO_4, 8.1 mmol L^{-1} Na_2HPO_4, pH 7.4), and with a ratio of 1:1 divided in appropriate aliquots and stored at −80 °C until further analysis.

Biochemical methods

SOD activity

SOD activity was determined by the method of Misra and Fridovich (1972), which is based on the ability of superoxide dismutase to inhibit the process of epinephrine self-oxidation in alkaline medium. In the reaction of colored adrenochrome formation, the superoxide anion-radical is formed as an intermediate product. Erythrocyte SOD activity was measured by monitoring the increase in the absorbance at 480 nm.

CAT activity

CAT was determined in erythrocytes by the method of Aebi (1984). To 3 mL H_2O_2 (54 mM H_2O_2 in 50 mM phosphate buffer, pH 7.0), 5 µL of a catalase solution was added and the decrease in H_2O_2 was measured spectrophotometrically (Beckman DU 500, USA) at 240 nm for 60 s at 25 °C. In the erythrocyte preparations, hemolysates were centrifuged (9000 *g*) and estimation of activity was made with 1% hemolysates. One unit of catalase activity was defined as the activity required for degrade 1 µmol hydrogen peroxide in 60 s.

GSH-Px activity

GSH-Px activity of erythrocytes was measured using hydrogen peroxide as substrate (Carlberg & Mannervik, 1972). Potassium azide was added to inhibit catalase. Potassium ferricyanide was added to inhibit the pseudo-peroxidase activity of hemoglobin. Conversion of NADPH was monitored continuously in a spectrophotometer at 340 nm for 3 min at 25 °C.

MDA level

The content of MDA was measured in plasma preparations by the thiobarbutiric acid (TBA) method which was modified from methods of Satoh (1978) and Yagi (1984). Peroxidation was determined as the production of MDA that, in combination with TBA, forms a pink chromogen compound whose absorbance at 532 nm was measured.

Cytogenetic analysis

SCE assay and proliferation index

With the aim of providing a better visualization of SCEs, 5-bromo-2-deoxyuridine (Sigma®, St. Louis, MO, final

concentration 20 mM) was added after culture initiation. The cultures were incubated in complete darkness for 72 h at 37 °C. Exactly 70 h and 30 min after beginning of the incubations, colcemid (Sigma®) was added to the cultures to achieve a final concentration of 0.5 mg L^{-1}. After hypotonic treatment (0.075 M KCl) followed by three repetitive cycles of fixation in methanol/acetic acid solution (3.1, v/v), centrifugation and resuspension. The cell suspension was dropped on to chilled and grease-free microscopic slides, air-dried, aged and then differentially stained for inspection of the SCE rate according to the fluorescence plus Giemsa (FPG) procedure (Perry & Wolff, 1974). For each treatment condition, 25 well-spread second division metaphases were scored and the values obtained were calculated as SCEs per cell.

In addition to SCEs, cells were analyzed for the relative frequency of first-division metaphases (M1; identifiable by uniform staining of both sister chromatids), second-division metaphases (M2; identifiable by differential staining of the sister chromatids), and third- and subsequent division metaphases (M3; identifiable by nonuniform pattern of staining). PI is the average number of replications completed by metaphase cells and is calculated as follows:

$$\mathrm{PI} = 1 \times (\%\ \mathrm{M1}) + 2 \times (\%\ \mathrm{M2}) + 3 \times (\%\ \mathrm{M3})/100.$$

MN assay

The MN test was performed by adding cytochalasin B (Sigma®; final concentration of 6 μg mL^{-1}) after 44 h of culture. At the end of the 72 h incubation period, the lymphocytes were fixed with ice-cold methanol acetic acid (1:1). The fixed cells were put directly on slides using a cytospin, and stained with May Grünwald-Giemsa. All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 2000 binucleated lymphocytes were examined per concentration (two cultures per concentration) for the presence of one, two or more micronuclei.

Comet assay

The comet assay also known as single cell gel electrophoresis (SCGE) was performed and scored according to Singh et al. (1988), Banu et al. (2001) and Prabhavathy Das et al. (2006). The cultures were set up by incubating lymphocytes for 72 h with *C. islandica* in diabetic groups. The control cultures were set up by incubating lymphocytes with the phosphate buffered saline (PBS) (at a final concentration of 1%). Ten milliliters of the 100 mL aliquots of the lymphocytes treated as above along with untreated samples were mixed with 120 mL of 0.5% low melting agarose and layered on the surface on glass slides previously coated with 140 mL of 1% normal melting agarose. After the application of cover slips, the slides were allowed to gel at 4 °C for 20 min. After carefully removing the cover slips, a second layer of 0.5% low-melting agarose was pipetted on to the slides and allowed to gel for a further 20 min at 4 °C. The slides were immersed in freshly prepared coldly lysing solution and refrigerated overnight followed by alkali treatment, electrophoresis and neutralization. The dried slides were then stained using silver nitrate solution after appropriate fixing. The whole procedure was carried out in dim light to minimize artifactual. DNA damage analysis was performed at a magnification of 100× using a light microscope after coding the slides. A total of 100 cells were screened per slide. A total damage score for each slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades (giving a maximum possible score of 400, corresponding to 100 cells at grade 4).

Statistics

The statistical analysis of experimental values was performed by one-way analysis of variance (ANOVA) and Duncan's test using the SPSS 13.0 software the level of 0.05 was regarded as indicative of statistical significance for all tests

Results

The effects of *C. islandica* on biochemical parameters in blood cultures of diabetic patients are present in Table 1. As compared with the controls, the activity of SOD, CAT and also GSH-Px enzymes were markedly decreased in erythrocytes of patients with T1D while MDA increased ($p < 0.05$). In the alone group, the *C. islandica* extract significantly increased the level of antioxidant enzymes at both dosage (5 and 10 μg mL^{-1}) but the effect of extract on the parameters was not dose related. As presented in Table 1, lichen extracts did not affect the MDA level measured in the human blood plasma. Noteworthy, *C. islandica* brought the antioxidant activity of erythrocytes closer to the control levels in blood cultures with T1D. The increase of antioxidant capacity was significant statistically in diabetic groups ($p < 0.05$) and MDA level returned to the control levels. However, the effect of extract on the MDA was not dose related (data not shown) (Table 1).

The frequency of MN, SCEs, PI and the rate of primary DNA damage (SCGE) in human lymphocytes were given in Figures 1–4, respectively. In T1D + *C. islandica* groups, separately and together, it was determined that the values of MN were near to control groups ($p > 0.05$) (Figure 1). Diabetes caused to generate a significant SCE rate in human lymphocytes as compared with controls ($p < 0.05$). We also compared SCE frequency in controls with and without *C. islandica* and did not find any significant difference in the frequency of SCE. On the contrary, *C. islandica* at both doses reduced the SCE frequency, but the reduction of the SCEs by lichen extract was sought in the blood with T1D ($p > 0.05$) (Figure 2). Regarding PI values, our results showed that treatments with *C. islandica* alone did not alter PI values compared to the control. Whereas, a statistically important decrease in the rate of PI was observed in patients with DM. However, the treatments with *C. islandica* (at both concentrations) increased the rate of PI as compared to the group with T1D alone (Figure 3). In the present study, comet assay as DNA damage analysis was performed and quantified with regard to diabetes and *C. islandica* administration (Figure 4). It was shown that the level of primary DNA damage significantly increased with T1D ($p > 0.05$), whereas there were no differences between controls and *C. islandica* groups. At the concentrations of *C. islandica*, it was clearly being seen that the rate of DNA damage was significantly decreased

Table 1. The effects of *C. islandica* extract on biochemical parameters in healthy or diabetic human blood cultures.

Groups#	SOD (U mL^{-1})	CAT (U g Hb^{-1})	GSH-Px (U mL^{-1})	MDA (μmol L^{-1})
Control (C)	101.8 ± 6.90^{b}	234.2 ± 7.68^{b}	90.8 ± 8.27^{b}	355.4 ± 12.99^{a}
Diabetes(T1D)	51.6 ± 4.97^{c}	137.6 ± 5.58^{c}	52.4 ± 6.34^{c}	825.4 ± 17.07^{b}
C. islandica 5 μg mL^{-1}	117.1 ± 7.36^{a}	248.4 ± 6.8^{a}	107.9 ± 7.62^{a}	362.7 ± 10.54^{a}
T1D + *C. islandica* 5 μg mL^{-1}	92.7 ± 5.26^{b}	219.4 ± 8.22^{b}	82.40 ± 4.87^{b}	374.6 ± 11.56^{a}

Different letters within each column present a statistical difference by Duncan multiple range test at $p < 0.05$. Unit definition for SOD (U mL^{-1}): one unit inhibits by 50% the maximum reduction of nitro blue tetrazoliumat 25 °C, pH 7.0; for CAT (U g Hb^{-1}): one unit decomposes one micromole of hydrogen peroxide per minute at 25 °C, pH 7.0; for GSH-Px (U mL^{-1}): one unit catalyzes the oxidation by H_2O_2 of 1.0 μmol of reduced glutathione to oxidized glutathione per min at pH 7.0 at 25 °C; MDA (μmol L^{-1}).
#Values are presented as mean ± S.D.; $n = 8$.

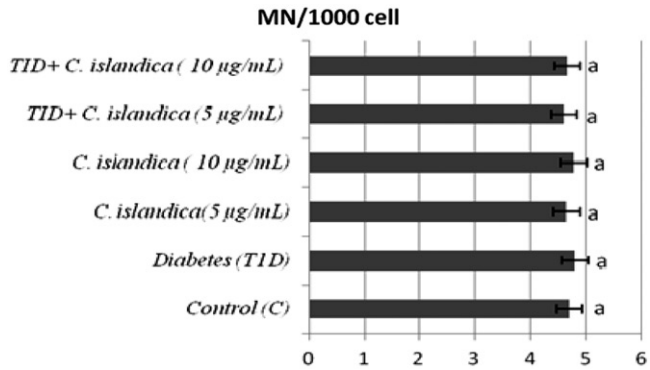

Figure 1. The frequencies of MN in cultured human lymphocytes from diabetic patients and control groups. Values are means ± standard deviation; $n = 8$. Different letters within each bar present a statistical difference by Duncan multiple range tests at $p < 0.05$.

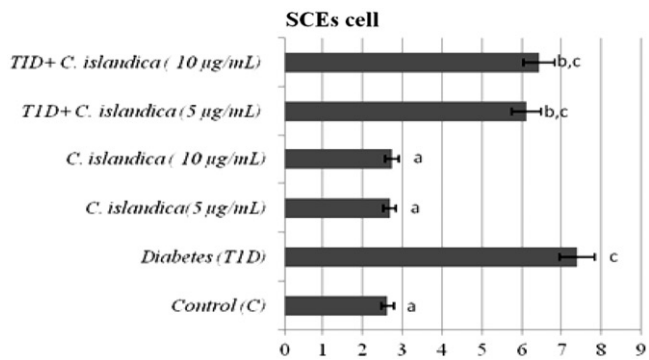

Figure 2. The frequencies of SCEs in cultured human lymphocytes from diabetic patients and control groups. Abbreviations are as defined in Figure 1.

against T1D ($p < 0.05$). Thus, *C. islandica* was established to display beneficial effects on DNA, by returning these values close to those of the control group after T1D disease. However, the lichen extract did not show any evidence of dose-related effect (data not shown).

Discussion

This study provides the first evidence that *C. islandica* has a protective role against T1D-induced oxidative stress and genomic instability in human blood. For T1D is an important observation that this disease increases oxidative stress in tissues (Perez-Gutierrez & Damian-Guzman, 2012; Tan et al., 2007). Our results are in agreement with previous studies in which T1D increases plasma level of MDA, an important

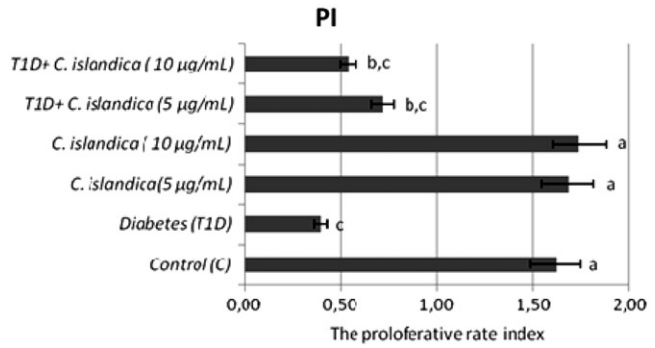

Figure 3. The rates of PI after treatment with *C. islandica* extract on human lymphocytes. Abbreviations are as defined in Figure 1.

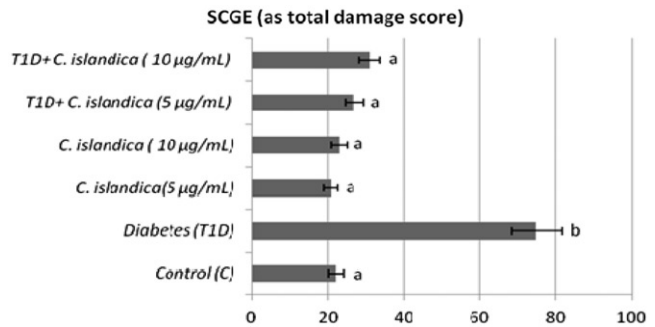

Figure 4. The effects of *C. islandica* extract on diabetic patients DNA damage determinated by SCGE assay in lymphocytes. Abbreviations are as defined in Figure 1.

biological marker of lipid peroxidation (Bulum & Duvnjak, 2011; Yousefi et al., 2011). And it is suggested that the positive relationship between MDA and T1D is significant because disease leads to increased production of ROS and it may also involve lipid peroxidation (Országhová et al., 2009). Under these conditions, diabetes may induce vascular dysfunction and hypertension (Naka et al., 2012). On the other hand, lichens has been used for centuries in traditional medicine and, especially over the last decade, many works dealing with lichens have been published, but it still is a matter of investigation by researchers of different properties (Valente et al., 2011). Those are the reasons why we decided to investigate the role of *C. islandica* in both the effects on oxidative stress of T1D and its effect on DNA damages in blood cells. The human body possesses a range of antioxidant components, which are distributed among biological fluids, tissues and cell compartments (Simões-Ambrosio

et al., 2010). Both enzymatic and non-enzymatic antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infectious and degenerative diseases (Nader et al., 2010). The oxidative stress develops when the levels of antioxidants are lowered (Tapiero et al., 2004). We have considered whether the effectiveness of *C. islandica* in response to T1D is associated with the induction of antioxidant activity. Our findings revealed that there were statistically significant differences in MDA level between the T1D group and T1D + *C. islandica* group. In the current study, it was also demonstrated that lichen extract administration in T1D group significantly decreased the level of MDA without depending on the dose. From previous reports, *C. islandica* extract determined adaptogenic-antistress effects, confirmed by its actions on oxidative stress parameters as MDA in laboratory animals (Cernescu et al., 2011b). Recent studies suggested a strong antioxidant effect of lichen extract that could ameliorate oxidative stress (Kosanic & Rankovic, 2011). The effects of antioxidants are pronounced in erythrocytes because these cells have increased amounts of hemoglobin and iron ions and consequently they are under higher oxidative stress (Vitturi et al., 2013). The results obtained *in vitro* suggest that the membrane fragility increases under oxidative stress conditions for the patient RBCs and the protection effect of antioxidants is due to their antioxidant properties (Moreira et al., 2011).

The protective effect of *C. islandica* can be explained by both the direct scavenging of free radicals produced by the indirect effect and the activation of oxidative repair enzymes. Gülçin et al. (2002) have reported that *C. islandica* has strong superoxide radical scavenging activity on peroxidation of linoleic acid. Thus, authors have underlined the occurrence of alterations in antioxidants upon the administration of *C. islandica*. It is known that the phenolic compounds of lichen may contribute directly to antioxidative action (Duh et al., 1999). And these compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables (Tanaka et al., 1988). In the present study, *C. islandica* had a pronounced effect on the T1D. In our study, some important key enzymes, such as erythrocyte SOD, CAT and GSH-Px can be involved in the inhibitory effect of ROS generation, which shall be further investigated. In previous studies erythrocyte SOD and GSH-Px activities were measured. The administration of *C. islandica* concluded to increase activities of these enzymes and thereby to result in the alleviation of oxidative stress (Kotan et al., 2011).

As known, antioxidant capacity comes from non-enzymes like glutathione (GSH), as well as enzymes such as SOD, CAT and GSH-Px (Murri et al., 2010). T1D included vascular abnormalities, associated with the reduction of SOD, CAT and GSH-Px activities (Yang et al., 2013). SOD is the primary step of the defense mechanism in the antioxidant system against oxidative stress. It catalyzes the dismutation of superoxide radicals (O_2^-) into molecular oxygen (O_2) and H_2O_2 (Kakarla et al., 2005). CAT activity is decreased in erythrocytes during T1D and its activity is inhibited by the superoxide radical (Shailey & Basir, 2012). The suppression of these enzyme activities has been linked to induction of oxidative stress (Stoppa et al., 2006). In addition, the observed decreases in GSH-related enzyme activities indicate that diabetes may induce oxidative stress in human blood by altering GSH metabolism (Yavari & Azizova, 2012). GSH-Px can protect DNA and lipids of the cell against the peroxidation products (Han, 2011; Kostic et al., 2007). The observed decline in the activity of GSH-Px in the blood of DM patients may be ascribed to the reduction in the level of the GSH and an increase in the level of peroxides (Al-Shebly & Mansour, 2012). GSH is one of the most abundant intracellular antioxidants in animal cells. Since it is considered to be one of the most abundant tripeptides in human organism and its action against oxidative radicals is well known. Thus, the balance of GSH-Px enzyme system may also be essential to remove superoxide anion and peroxides generated in erythrocytes (Allen & Bradley, 2011).

The enzyme inactivations induced by T1D also corroborate by the genotoxic findings. The results obtained by us indicate a significant increase in the ratios of the SCEs in lymphocytes, which is in accordance with the previous reports (Cinkilic et al., 2009; Sheth et al., 2006). The SCEs are formed by toxic oxygen metabolites in cultured human leukocytes and other mammalian cells (Madrigal-Santillán et al., 2010). T1D is related to LPO and oxidation of DNA *in vivo* and *in vitro* (Chistiakov et al., 2012). So this disease could contribute to the formation of the genome leading to carcinogenesis (Dobrila-Dintinjana et al., 2012). Lipid peroxides enter the nucleus where they react with Fe^{+2} to generate the alkoxyl radical which attacks DNA (Fraga & Tappel, 1988). Also, intracellular calcium levels increase as a result of oxidative damage to cell membranes, calcium then enters the nucleus where it can activate nucleases which cause DNA strand breaks (McConkey et al., 1989). DNA damage and defective DNA repair cause SCEs (Dumitrache et al., 2011). In this study, the abnormal signs of T1D on blood cells were dramatically and dose-dependently unchanged by *C. islandica*. Hence the rates of SCEs were not unfortunately fully decreased in lymphocyte cells.

MNs are the results of acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase of the mitotic cells (Albertini et al., 2000). In the present study, there were no differences in MN frequencies in the lymphocytes of T1D patients when compared with controls. Our current data confirm and expand previous findings by showing that patients with T1D have unchanged levels of MNs (Cinkilic et al., 2009).

Notably, previous studies of oxidative damage in DM patients have used tests, including the comet assay, to investigate DNA damage (Ross, 2011; Sardas et al., 2001). Diabetic patients have increased DNA damage as identified by the comet assay, and this damage may cause an increase in the risk of cancer (Bonassi et al., 2007). Our results showed a significant increase in the ratios of the primary DNA damage in lymphocytes in accordance with the previous reports. Investigators reported oxidative DNA damage in the comet assay in both type-1 and type-2 diabetic patients (Palazzo et al., 2012; Sardas et al., 2001). Moreover, these authors reported that DNA damage as observed in the comet assay was high in individuals with T1D. The comet assay has been proposed to be the most sensitive procedure for detecting DNA fragmentation. Due to its simplicity and sensitivity, the comet assay has rapidly gained acceptance as a genotoxicity assay. Additionally, the

comet assay has shown that strand breaks arise from DNA damage generated by oxidative stress. Furthermore, the comet assay has been found to be technically suitable for the routine measurement of DNA damage (Moller et al., 2000). Our study revealed that *C. islandica* extract significantly decreased the ratios of the primary DNA damage when the values were comparable with that of the T1D groups.

Growing evidence suggest that one of causes of increased level of oxidative DNA damage in diabetic patients is reduced antioxidant defense system (Piwowar et al., 2007). The biologically fundamental macromolecules such as nucleic acids and proteins in mammalian cells defend themselves with antioxidants (Kedziora-Kornatowska et al., 2004). At this point, our results showed that lichen extract exhibited antigenotoxic properties at concentrations ranged at 5 and 10 $\mu g\,mL^{-1}$ concentrations. *C. islandica*, as mentioned above, supported the antioxidant defense mechanism against T1D. The results obtained in the present study elucidated that DM also induced a reduction in PI in human lymphocytes. These data were similar to the findings from earlier studies (Varga et al., 2011). PI has been used for the assessment of the cytostatic action of various therapeutic agents (Baka et al., 2009). *C. islandica* was useful and increased the PI, which was decreased by diabetes.

Conclusion

The biochemical and genotoxic tests dealing with *C. islandica* are used frequently in the food industry reveal safe results for a food supplement. The present study is the first report describing *C. islandica*'s ability to protect nuclear DNA from oxidative damage against T1D and it is speculated that protective effects of lichen extract are a consequence of its ability to reduce MDA formation and increase associated antioxidants. In conclusion, we suggest that it may be a chemopharmaceutical molecule of interest against T1D.

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Declaration of interest

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