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Research Article

The Effects of Lichens Extracts in The Healthy Rats and The Medical Utility of These Extracts in The Prevention of Diabetes-Associated Multiple Organ Failures

Suat ÇOLAK [1] Fatime GEYIKOĞLU [2] Hasan TURKEZ [3] Murat BAKIR [2]
Mirkhalil HUSSEINIGOUZDAGANII [2] Serpil CAN [4] Gülşen ÇIĞŞAR [5] Ali ASLAN [6]

[1] Artvin Coruh University, Department of Biology, Faculty of Science and Arts, TR-08100 Artvin - TÜRKİYE
[2] Atatürk University, Department of Biology, Faculty of Science, TR-25100 Erzurum - TÜRKİYE
[3] rzurum Technical University, Department of Molecular Biology and Genetics, Faculty of Science, TR-25100 Erzurum - TÜRKİYE
[4] Kafkas University, Department of Physiology, Faculty of Medicine, TR-36100 Kars - TÜRKİYE
[5] Kafkas University, Department of Emergency Medical, Faculty of Medicine, TR 36100 Kars - TÜRKİYE
[6] Atatürk University, Department of Biology, Kazim Karabekir Education Faculty, TR-25100 Erzurum - TÜRKİYE

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Abstract

In the present study, we firstly assessed *Cetraria islandica* and Pseudevernia furfuracae to avoid detrimental effects on multiple tissues of rats. Diabetes mellitus (DM) with the subsequent generation of oxidative stress represents a major risk factor for organs. The second aim of this study is to investigate whether administration of both lichens could prevent type 1 diabetes (T1D)-induced organ dysfunctions. During two weeks, both control and diabetic rats were treated with aqueous lichen extracts. The metabolic changes were determined. On day 14, after animals were decapitated, required samples for biochemical and genetic analysis were collected. Oxidative damage of DNA was estimated by measuring the increase in 8-hydroxy-2′-deoxyguanosine formation. Biochemical parameters were used to observe and evaluate the functional changes in tissues. Experimental data showed that the increasing doses of lichens alone have not any detrimental effect on above parameters. Moreover, *C. islandica* decreased the diabetes-induced glucose and malondialdehyde (MDA) levels. Thus, it seemed that the antioxidant treatment has an important effect on the organ failure in ill rats. However, the protective effect of *C. islandica* was inadequate on diabetes-induced disorders and DNA damages. Lichens are a safe in the studied dose range but the power of *C. islandica* is limited because of intensive oxidative stress in essential organs of T1D rats.

Keywords: *Cetraria islandica, Pseudevernia furfuracae, Diabetes mellitus, Organ dysfunctions, Antioxidant capacity, 8-hydroxy-2′-deoxyguanosine*

Liken Ekstrelerinin Sağlıklı Ratlar Üzerindeki Etkileri ve Bu Ekstrelerin Diyabete Bağlı Çoklu Organ Yetmezliğinin Önlenmesinde Medikal Kullanımı

Özet

Bu çalışmada, ilk olarak *Cetraria islandica* ve *Pseudevernia furfuracae* türü liken ekstraktlarının sıçanlara ait çeşitli dokular üzerine zararlı etkilerinin olup olmadığı değerlendirilmiştir. Diabetes mellitusa (DM) bağlı oksidatif stres, organlar için büyük bir risk faktörü oluşturmaktadır. Çalışmamızın ikinci bölümünde ise her iki liken ekstaktı uygulamasının tip 1 diyabet kaynaklı organ bozukluklarına karşı koruyucu olup olmadıklarını araştırılmıştır. İki hafta boyunca hem kontrol hem de diyabetli ratlara sulu liken ekstreleri uygulanmıştır. Süre sonunda metabolik değişimler belirlenmiştir. Ondördüncü günde, hayvanların yaşamları servikal dislokasyonla sonlandırıldıktan sonra biyokimya ve genetik çalışmalar için gerekli örnekler alınmıştır. DNA'nın oksidatif hasarı 8-hidroksi-2′-deoksiguanosin oluşumunda meydana gelen artış ölçülerek hesaplanmıştır. Biyokimyasal parametreler dokularda meydana gelen fonksiyon değişimlerinin gözlenmesi ve değerlendirilmesi için kullanılmıştır. Deney sonuçları tek başına uygulanan liken ekstraktlarının artan dozlarda herhangi bir zararlı etkiye sahip olmadığını göstermiştir. Ayrıca, *C. islandica* diyabete bağlı artan kan glikoz ve malondialdehit (MDA) seviyesini kısmen düşürmüştür. Antioksidan tedavinin hasta ratlarda organ yetmezliğine karşı fayda sağlayacağı düşünülmektedir. Bununla birlikte, *C. islandica*'nın antioksidan etkisinin diyabet teşvikli bozukluklarda ve DNA hasarına karşı yetersiz olduğu anlaşılmıştır. Sonuç olarak, likenlerin çalışılan doz aralığında güvenli olduğu ve tip 1 diyabetin neden olduğu yaşamsal organlar üzerindeki güçlü oksidatif stres nedeniyle *C. islandica* sınırlı bir etkiye sahip olduğu gözlenmiştir.

Anahtar sözcükler: *Cetraria islandica, Pseudevernia furfuracae, Diyabetes mellitus, Organ bozuklukları, Antioksidan kapasite, 8-hidroksi-2′-deoksiguanosin*

İletişim (Correspondence)
+90 442 2314325
geyikogluff@yahoo.com

INTRODUCTION

DM is one of the most common endocrine metabolic disorders in developing countries. T1D affects millions of people worldwide and has an incidence that is increasing at a striking rate, especially in young children [1]. A large body of earlier investigations has examined the role of oxidative stress in insulin resistance in type 1 and type 2 diabetes and its associated complications [2]. The investigations have revealed that hyperglycemia degrades antioxidant enzyme defenses by allowing reactive oxygen species to damage cells and tissues [3]. The balance of glucose by the drugs has emerged as a novel therapeutic approach to disease that develop with high circulating glucose. Lichens have been used for various purposes such as dyes, perfumes and remedies in folk medicine indicating the pharmaceutical potential of lichens [4]. The lichens have also antioxidant, antimicrobial and anticancer properties [5,6]. It is documented that they effective in the treatment of tuberculosis [7], hemorrhoids and dysentery [8] and also induce apoptosis in colon [9,10] and prostate cancers [11]. Lichen species are very common in Turkey. Unique lichen flora has attracted many researchers on the systematical basis [12]. It is pointed that lichens may be easily accessible sources of natural drugs that could be used as a possible food supplement or in pharmaceutical industry [13].

Diabetic nephropathy (DN) remains the most common cause of end-stage renal disease [14]. Chronic kidney disease (CKD) is characterized by progressive decline in renal function [15]. To prevent the development of this disease and to improve advanced kidney injury, effective therapies are required. Although diabetic hepatopathy is potentially less common, it may be appropriate for addition to the list of target organ conditions related to diabetes [16]. Enzymology is a diagnostic indicator for diabetes in liver dysfunctions. Therefore, liver function tests (LFTs) are commonly used in clinical practice to screen the progression of disease, and monitor the effects of hepatic drugs. The most common LFTs include the serum aminotransferases as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). A rational therapy rich in antioxidants reduces levels of metabolical disorders in diabetic individuals [17]. Here we used a strategy based on feasible alternative therapeutic for metabolically labile patients with T1D. So far, lichens have not been evaluated under controlled conditions suggesting improved glucose control and a reduced risk of hypoglycemia. 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 two well-known lichen species *Cetraria islandica (C. islandica)*, *Pseudevernia furfuracea (P. furfuracea)* in relation to the survival of liver, pancreas and kidney in model with T1D. In many countries, *C. islandica* is used medicinally e.g. for colds, bronchitis, and asthma [18] and *P. furfuracea* is studied for antioxidant properties *in vitro* conditions [19].

Although these lichens are popular around the world, their beneficial and/or adverse effects on human health have not been scientifically determined yet. Hence, our study firstly assessed *C. islandica* and *P. furfuracea* extracts to avoid detrimental effects on multiple tissues of rats in vivo conditions. Secondly, the aim of present study was to evaluate the effects of *C. islandica* and *P. furfuracea* on the development of diabetes-related organ dysfunctions, in relation to biochemical changes and also DNA damages in diabetic rats. The correlation of DNA damage with adverse health effects is important in evaluating the safety of various agents and prospective therapeutics. Many techniques exist that afford the ability to identify and measure cellular DNA damage upon exposure to a suspected genotoxic agent; however, the modified nucleoside 8-hydroxy-2'-deoxyguanosine (8-OHdG) is commonly used as a reliable and sensitive index of oxidative DNA damage [20].

There is a very little information regarding the biological activity of these two lichen species. Therefore, in this study, the effects of two lichen species at the different doses (250 and 500 mg/kg bw) were investigated in non-diabetic, diabetic and lichen-treated diabetic rats.

MATERIAL and METHODS

Lichen Extracts

Lichen species, *C. islandica* (L.) Ach. and *P. furfuracea* (L.) Zopf were collected from the Giresun, Erzurum and Artvin province in Turkey, during summer of 2011. The samples were identified using various flora books and papers [12,21]. Identified samples were air-dried and stored in the herbarium of Kazım Karabekir Education Faculty, Atatürk University. For water extraction of lichenes, 20 g sample was mixed with 400 mL distillated and boiling water using magnetic stirrer for 15 min. Then the extracts were filtered over Whatmann No. 1 paper. The filtrates were frozen and lyophilized in lyophilizator (Labconco, Freezone IL) [19].

Animals

Seventy adult male Spraque-Dawley rats (6 weeks old, weighing 200-250 g) obtained from Medical Experimental Application and Research Center, Atatürk University were used. Animals were housed inside polycarbonate cages in an air-conditioned room (22±2°C) with 12-h light-dark cycle. Standard rat feed and water were provided ad libitum. The rats were allowed to acclimatize to the laboratory environment for 7 days before the start of the experiment. All procedures were performed in conformity with the Institutional Ethical Committee for Animal Care and Use at Atatürk University (*Protocol Number*: ATADEM, B.30.2.ATA.0.23.85-11 and Date: 26.03.2010) and the Guide for the Care and Use of Laboratory Animal.

Experimental Induction of Diabetes

Streptozotocin (STZ) (single dose of 50 mg/kg bw) (purchased from Sigma, St Louis, MO, USA) dissolved in freshly prepared 0.01 M citrate buffer pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg. STZ-induced animals exhibited massive glycosuria and hyperglycemia within 2 days. T1D was confirmed in STZ-induced rats by measuring the fasting blood glucose concentration 72 h after the injection of STZ. The rats with blood glucose level>200 mg/dl were considered to be diabetic and were used in the experiment [22].

Experimental Design

There was very little evidence in the literature about lichens and their uses on therapy and therefore the study included from 5 to 500 mg/kg large-dose range. Use of large-dose range of lichens provided a more convenient and potentially more effective strategy for assisting diabetic recovery. The effective doses were 250 and 500 mg/kg and thus the rats were randomly divided into ten groups (n=7) as follows *(Table 1)*:

The aqueous extracts of lichens were administered intraperitoneally daily for 14 days. After completion of the experiments, rats were killed via rapid decapitation without anaesthetic. The body weight, feed, and water intake in animals were measured. The blood and tissue samples of rats were collected for biochemical and oxidative DNA damage analyses.

Biochemical Analyses

The blood glucose levels were measured with a automatic glucometer (®Accu-Chek GO) in a tail blood before decapitation. The rat serum insulin levels were detected by commercial elisa kit purchased from Randox Laboratories Ltd Dialab Produktion und laborinstriementen Gessellschaftm.b.H. (A-1160 Wien-panikengasse, Germany). The serum samples were prepared by centrifugation at 1.600 g at 4°C for 15 min using a cooling centrifuge (Nüve, NF 400R, Turkey). The blood glucose and serum insulin levels were respectively expressed in mg/dL and µg/L.

Blood samples were collected into serum superetor tubes (Microtainer; Becton Dickinson, Franklin Lakes, NJ, USA), allowed to stand (75-90 min), centrifuged (11.000 g, 5 min), serum harvested, and stored at -20°C. The following parameters for liver injury were measured by an automated biochemical analyzer (OLYMPUS AU 2700) with Bayer testing kits (Bioclinica): AST, ALT and LDH. Renal impairment was assessed by blood urea nitrogen (BUN) and serum creatinine (Cr) and uric acid (UA) levels with an autoanalyzer (Syncron LX 20, Galway, Ireland) by using commercial Beckman Coulter diagnostic kits. After the 14 day treatment, the rats were fasted overnight and then decapitated. Thoracic cavities were incised open; pancreas, liver, and kidney tissues were removed and stored at −70°C. All defrosted tissues were homogenized in ice shower containing 4 mL of 0.2 M phosphate buffer at pH 7.4. Homogenates were centrifuged at 3.000 g for 15 min at 4°C to remove tissue remnants. Activities were determined in the supernatant. Total antioxidant capacity (TAC) levels were measured using the commercial kit (Rel Assay Diagnostic) Syncron LX autoanalyzer [23] Lipid peroxidation was determined by quantifying malondialdehyde (MDA) concentrations, which was spectrophotometrically measured by the absorbance of a red-colored product with thiobarbituric acid [24].

Nucleic Acid Oxidation

DNA was extracted from 100 mg tissue samples by commercially available DNA extraction kits (DNAzol® Life Technologies, Gaithersburg, MD, USA). Via using these kits up to approximately 5 µg of DNA/mg of tissue was obtained. Extracted DNA was dissolved in water at 1-5 mg/mL. Then DNA samples were converted to single-stranded DNA by incubating the sample at 95°C for 5 min and rapidly chilling on ice, and digested to nucleosides by incubating the denatured DNA with 5-20 units of nuclease P1 for 2 h at 37°C in 20 mM sodium acetate, pH 5.2, and following with treatment of 5-10 units of alkaline phosphatase

Table 1. *Experimental groups*
Tablo 1. *Deney grupları*

Non-diabetic	**Non-diabetic control**	**Group 1 (NC):** Non-diabetic control rats (n=7)
	Treated with CIAE	**Group 3 (CIAE-250):** Rats recevied 250 mg/kg bw aqueous extract *of Cetraria islandica* (n=7)
		Group 4 (CIAE-500): Rats recevied 500 mg/kg bw aqueous extract *of Cetraria islandica* (n=7)
	Treated with PFAE	**Group 5 (PFAE-250):** Rats recevied 250 mg/kg bw aqueous extract *of Pseudevernia furfuracea* (n=7)
		Group 6 (PFAE-500): Rats recevied 500 mg/kg bw aqueous extract *of Pseudevernia furfuracea* (n=7)
Diabetic	**Diabetic control**	**Group 2 (DC):** Diabetic control rats recevied 50 mg/kg bw single injection (ip) of STZ (n=7)
	Treated with CIAE	**Group 7 (CIAE-250):** Diabetic rats treated with 250 mg/kg bw aqueous extract *of Cetraria islandica* (n=7)
		Group 8 (CIAE-500): Diabetic rats treated with 500 mg/kg bw aqueous extract *of Cetraria islandica* (n=7)
	Treated with PFAE	***Group 9 (PFAE-250):*** Diabetic rats treated with 250 mg/kg bw aqueous extract *of Pseudevernia furfuracea* (n=7)
		Group 10 (PFAE-500): Diabetic rats treated with 500 mg/kg bw aqueous extract *of Pseudevernia furfuracea* (n=7)

for 1 h at 37°C in 100 mM Tris, pH 7.5 [25]. The levels of 8-OHG in the samples were then quantiated using a HPLC technique with electrochemical detection (HPLC-EC) by an adaptation of the method of Kasai et al.[26] as described previously [27]. Briefly we used Kontron HPLC pump for 420 as HPLC apparatus, Beckman ultrasphere (0.46 x 25 cm) as column, and 8% aqueous methanol containing 10 mM NaH_2PO_4 as eluent, the flow rate was 1 ml/min. Peaks gained with electrochemical (for 8-OHdG) and UV (for dO) detectors were integrated under a background noise corection loaded on an integrator. The levels of 8-OHdG were determined as numbers of 8-OHdGs per l06 dOs, by calibration against curves from runs of standard samples, containing known amounts of authentic 8-OHdG and dG. During the asays, light and air contamination were avoided as strictly as posible.

Statistical Analysis

For statistical analysis, we used SPSS for Windows 13.0 (SPSS Inc., Chicago, USA). The experimental data were analysed using oneway analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons. Results are presented as mean ± standart deviation (SD) and values $P<0.05$ were regarded as statistifically significant.

RESULTS

The diagnostic parameters of both control and experimental rats before and after 14 days of treatment with STZ are shown in *Fig. 1* and *Fig. 2*. According to figures, the feed and water intake in diabetic rats are significantly increased throughout the study period as compared with the normal control group. Intraperitoneal administration of two lichen species extracts with low doses did not have a positive effect on diagnostic and body weight compared with diabetic rats. Moreover, similar results were also observed with higher doses administration of lichen extracts (data not shown). Conversely, the weight loss of ill animals is prominent.

In addition, intraperitoneal administration of STZ into the rats causes significant diabetogenic response in subjects with significant increase in the level of blood sugar and a significant decrease in the level of insulin as compared with normal rats (*Fig. 3* and *Fig. 4*). The blood glucose level is increased from an avarage 100 mg/dL to about 450 mg/dL. And the insulin level is decreased to 4 µg/L from 9 µg/L. Following intraperitoneal injection of *C. islandica* and *P. furfuracea* extracts, the feed and water intake are not changed as compared with the diabetic untreated rats. Furthermore, the extracts alone have not any adverse effect the body weight of the animals, blood glucose and insulin levels (data not shown). In animals with T1D, the treatment with *C. islandica* does not show positive effect on above parameters except for glucose and insulin levels. It is established that the administration of lichen at the dose 250 mg/kg, the blood glucose reduces and insulin levels increases as compared with diabetic rats ($P>0.05$). Depending on the increasing dose, the responses are not enhanced and such changes go unnoticed in T1D rats. Unfortunately, above parameteres remain unchange with respect to 250 mg/kg dose of *P. furfuracea* in diabetic animals and also both lichen extracts do not show any evidence of dose-related effect. Often such change goes unnoticed in pictures, although it can affect preference.

Table 2 shows the effects of *C. islandica* and *P. furfuracea* on biochemical parameters in all experimental groups. As compared with the controls, the TAC level are markedly decreased in pancreas, kidney and liver of diabetic rats

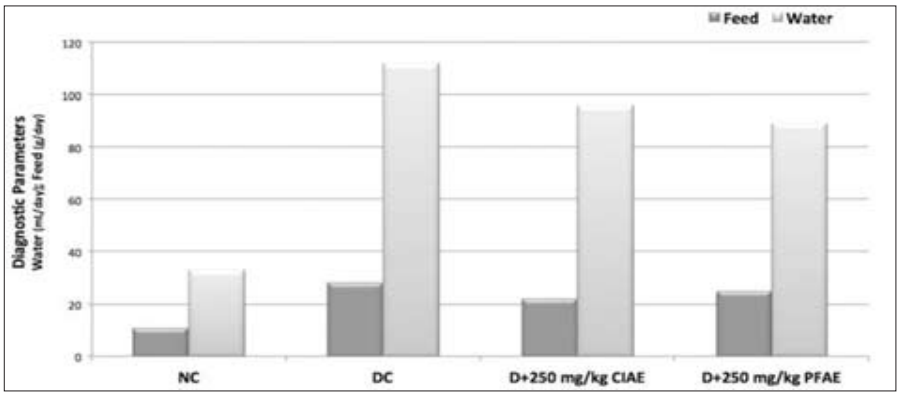


Fig 1. The effect of aqueous extracts of *C. islandica* (CIAE) and *P. furfuracea* (PFAE) on feed and water intake in control and diabetic rats (n=7); **NC:** Non-diabetic control group, **DC:** Diabetic control group, **D+CIAE:** Diabetic rats treated with *C. islandica* aqueous extract (250 or 500 mg/kg) and **D+PFAE:** Diabetic rats treated with *P. furfuracea* aqueous extract (250 or 500 mg/kg)

Şekil 1. Kontrol ve diyabetik sıçanlarda yem ve su alımı üzerindeki *C. islandica* (CIAE) ve *P. furfuracea* (PFAE) sulu ekstraklarının etkisi (n=7); NC: Non-diyabetik kontrol grubu, DC: Diyabetik kontrol grubu, D + CIAE: *C. islandica* sulu ekstresi ile tedavi edilen diyabetik sıçanlar (250 veya 500 mg/kg) ve D + PFAE islandica: *P. furfuracea* sulu ekstresi ile tedavi edilen diyabetik sıçanlar (250 veya 500 mg/kg)

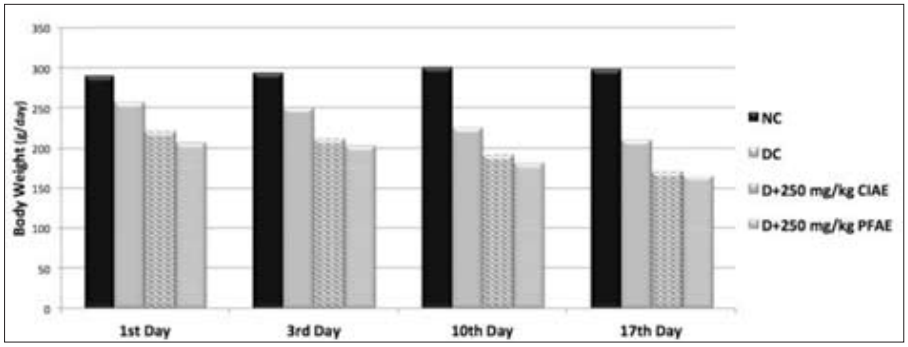
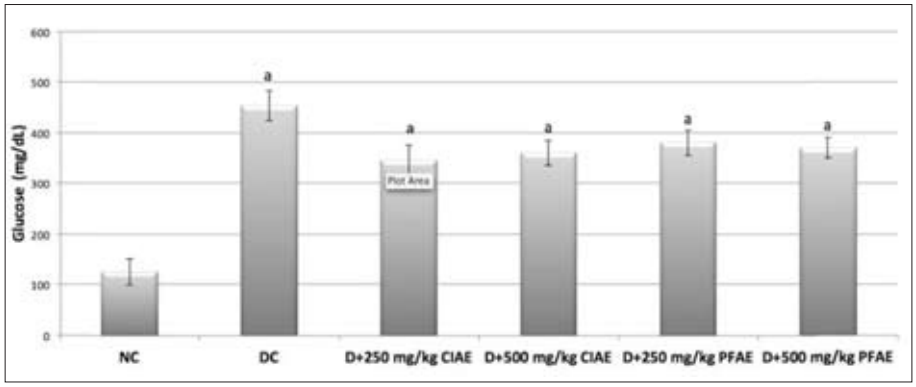


Fig 2. The effect of aqueous extracts of *C. islandica* (CIAE) and *P. furfuracea* (PFAE) on body weight in control and diabetic rats (n=7)

Şekil 2. *C. islandica* (CIAE) ve *P. furfuracea* (PFAE) sulu ekstraktlarının kontrol ve diyabetik sıçanların vücut ağırlığına etkisi (n=7)

Fig 3. The effect of aqueous extracts of *C. islandica* (CIAE) and *P. furfuracea* (PFAE) on blood glucose level in control and diabetic rats (n=7); Data are mean±SD values (n=7). They are significantly different between [a]NC and [b]DC group. (P>0.05; Tukey's multiple range test)

Şekil 3. *C. islandica* (CIAE) and *P. furfuracea* (PFAE) sıvı ekstraktlarının kontrol ve diyabetik sıçanların kan glukoz seviyesine etkisi (n=7); Veriler ort±SD değerlerdir (n=7). [a]NC ve [b]DC gruplar arasında anlamlı bir farklılık vardır. (P>0.05; Tukey çoklu karşılaştırma testi)



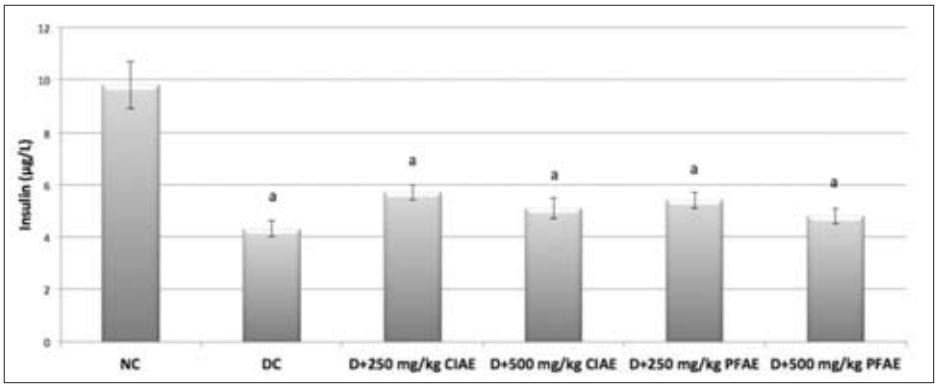


Fig 4. The effect of *C. islandica* (CIAE) and *P. furfuracea* (PFAE) extracts on serum insulin level in control and diabetic rats (n=7); Data are mean±SD values (n=7). They are significantly different between aNC and bDC group. (P > 0.05; Tukey's multiple range test)

Şekil 4. *C. islandica* (CIAE) and *P. furfuracea* (PFAE) ekstraktlarının kontrol ve diyabetik sıçanların serum insulin seviyelerine etkisi (n=7); Veriler ort±SD değerlerdir (n=7). [a]NC ve [b]DC gruplar arasında anlamlı bir farklılık vardır. (P> 0.05; Tukey çoklu karşılaştırma testi)

while MDA increased (P<0.05). In alone lichen treatments, MDA levels remain unchanged and lichens do not show any adverse effect with respect to increasing dose. Furthermore, the *P. furfuracea* extract increases the level of TAC at both dosage (250 and 500 mg/kg) but the best result is observed at the doses of *C. islandica*. However, the increase of TAC after supplementation with *C. islandica* is inadequate STZ-induced diabetic groups and MDA levels does not return to the control values (P>0.05). Moreover, the effect of extracts on these parameters is not dose related (data not shown).

In the rat kidney tissues, the levels of BUN and UA decrease in the *C. islandica* 250 and *C. islandica* 500 mg/kg groups when compared with the T1D *(Table 3)*; however, statistically significant differences were not seen between these groups and the control groups (P>0.05). Serum Cr is not reduced in T1D+*C. islandica* and T1D+*P. furfuracea* groups, whereas, all parameters are similar in experimental rats with *C. islandica* and *P. furfuracea* alone to controls.

In present study, activity levels of serum marker enzymes of liver are found elevated markedly in rats with T1D *(Table 4)*. No such changes are observed in control rat samples. As is evident from table, lichen doses alone (250 and 500 mg/kg) are not change the activity levels

Table 2. *The effect of C. islandica (CIAE) and P. furfuracea (PFAE) extracts on MDA and TAC levels in pancreas, kidney and liver tissues in normal and diabetic rats*

Table 2. *Kontrol ve diyabetik ratlarda pancreas, böbrek ve karaciğer dokuları TAC ve MDA seviyeleri üzerine C. islandica (CIAE) ve P. furfuracea (PFAE) ekstraktlarının etkisi*

Groups[Ψ] **(mg/kg bw)**	**MDA (nmol/g tissue)**			TAC (mmol Trolox Equiv/L)		
	Pancreas	**Liver**	**Kidney**	**Pancreas**	**Liver**	**Kidney**
Normal	4.33±0.97[a]	3.46±0.43[a]	6.61±0.32[a]	1.51±0.12[d]	1.81±0.76[d]	1.63±0.3[d]
Diabetic control	9.62±1.01[b]	8.02±0.85[b]	13.79±1.06[b]	0.79±0.17[f]	1.07±0.11[f]	0.94±0.13[f]
CIAE (250)	4.17±0.66[a]	3.04±0.69[a]	6.23±0.73[a]	1.95±0.16[a]	2.23±0.31[a]	2.14±0.49[a]
CIAE (500)	4.26±1.09[a]	3.28±0.29[a]	6.36±0.44[a]	1.79±0.29[b]	2.11±0.17[b]	1.97±0.29[b]
PFAE (250)	4.36±0.59[a]	3.35±0.21[a]	6.47±0.81[a]	1.71±0.19[b,c]	2.04±0.22[b,c]	1.87±0.34[b,c]
PFAE (500)	4.42±0.78[a]	3.51±0.75[a]	6.58±0.37[a]	1.63±0.28[c]	1.96±0.46[c]	1.79±0.26[c]
Diabetic+ CIAE(250)	9.17±0.85[b]	7.65±0.39[b]	13.39±1.52[b]	0.99±0.37[e,f]	1.26±0.43[e,f]	1.13±0.33[e,f]
Diabetic+ CIAE(500)	9.55±1.02[b]	7.93±0.46[b]	13.72±1.64[b]	0.83±0.32[f]	1.12±0.25[f]	1.0±0.28[f]
Diabetic+ PFAE(250)	9.59±0.88[b]	7.98±0.37[b]	13.76±1.73[b]	0.81±0.14[f]	1.11±0.21[f]	0.98±0.13[f]
Diabetic+ PFAE(500)	9.60±1.06[b]	8.01±0.54[b]	13.78±1.41[b]	0.80±0.24[f]	1.08±0.39[f]	0.95±0.19[f]

[Ψ]*Values are mean±SD of seven rats in each group (n=7). Means of five measurements marked by different letters within each column present a statistical difference at P<0.05 CIAE: C. islandica aqueous extract (250 or 500 mg/kg) and PFAE: P. furfuracea aqueous extract (250 or 500 mg/kg)*

Table 3.*The effect of C. islandica (CIAE) and P. furfuracea (PFAE) extracts on serum BUN, UA and Cr levels in normal and diabetic rats*

Table 3. *Kontrol ve diyabetik ratlarda serum BUN, UA ve Cr seviyeleri üzerine C. islandica (CIAE) ve P. furfuracea (PFAE) ekstraktlarının etkisi*

Groups[Ψ]	**BUN (U/L)**	**UA (U/L)**	**CRE (U/L)**
Normal	24.67±3.54	1.35±0.13	0.37±0.04
Diabetic control	91.11±9.17[a]	3.87±0.89[a]	0.84±0.08[a]
CIAE (250 mg/kg)	23.94±4.15[b]	1.17±0.54[b]	0.48±0.05[b]
CIAE (500 mg/kg)	24.60±2.42[b]	1.42±0.61[b]	0.39±0.02[b]
PFAE (250 mg/kg)	24.43±5.01[b]	1.21±0.49[b]	0.34±0.01[b]
PFAE (500 mg/kg)	23.27±7.16[b]	1.33±0.44[b]	0.36±0.02[b]
Diabetic+ CIAE (250 mg/kg)	86.41±6.94[a]	3.22±0.21[a]	0.79±0.06[a]
Diabetic+ CIAE (500 mg/kg)	90.17±6.82[a]	3.79±0.38[a]	0.81±0.07[a]
Diabetic+ PFAE (250 mg/kg)	91.56±7.01[a]	3.85±0.29[a]	0.82±0.08[a]
Diabetic+ PFAE (500 mg/kg)	91.87±5.37[a]	3.91±0.41[a]	0.85±0.07[a]

[Ψ]*Values are mean±SD of seven rats in each group (n=7).* [a]*Significant difference was detected in experimental groups comparing with normal group at P<0.05.* [b]*Significant difference was detected in experimental groups comparing with diabetic control group at P<0.05*

of AST, ALT and LDH (P>0.05). And the treatment with *C. islandica* can bring a decrease in the activity levels of these enzymes when compared to *P. furfuracea* group. Our results clearly reveal that *C. islandica* presents positive effects on the activities of enzymes without undepending on dose against T1D-induced liver damages. However, the levels of all ALT and AST enzyme samples do not show a significant similarity with control values after treatments with *C. islandica* in T1D. Moreover, the LDH enzyme activity is not normalized by doses of *C. islandica* exposure.

The status of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in studied tissues of control and experimental groups is presented in *Fig. 5*. Compared with the control group, there are significant increases in hepatic, kidney and pancreatic 8-OHdG levels in STZ-induced diabetic rats (P<0.05). Whereas, all doses of *C. islandica* and *P. furfuracae* extracts alone have not any effect on the level of 8-OHdG. However, post-treatment of *C. islandica* extracts can't significantly restore the oxidative DNA damage in the multiple tissues of diabetic rats without undepending on dose.

DISCUSSION

Increased throughput of any substrate involves a regional imbalance between ROS production and breakdown. Oxidative stress is a consequence of high circulating glucose levels in diabetic patients and rodents. Furthermore, a change in the balance of glucose substrate entails a degree of cellular oxidative stress [28]. In present study we assessed indicator of oxidative stress, MDA, in

Table 4. *The effect of C. islandica (CIAE) and P. furfuracea (PFAE) extracts on serum AST, ALT and LDH levels in control and diabetic rats*
Tablo 4. *Kontrol ve diyabetik ratlarda serum AST, ALT ve LDH seviyeleri üzerine C. islandica (CIAE) ve P. furfuracea (PFAE) ekstraktlarının etkisi*

Groups[Ψ]	**AST (U/L)**	**ALT (U/L)**	**LDH (U/L)**
Normal	167±26.57	51.15±6.27	1335.21±134.76
Diabetic control	371.1±43.63[a]	82.91±4.72[a]	2069.48±204.11[a]
CIAE (250 mg/kg)	172.9±24.71[b]	51.19±3.94[b]	1305.56±153.45[b]
CIAE (500 mg/kg)	174.3±27.91[b]	54.39±2.99[b]	1349.35±126.24[b]
PFAE (250 mg/kg)	169.7±35.07[b]	53.25±5.17[b]	1363.40±143.46[b]
PFAE (500 mg/kg)	170.5±24.03[b]	51.72±4.62[b]	1427.72±137.08[b]
Diabetic + CIAE (250 mg/kg)	316.4±36.24[a]	75.87±3.66[a]	1995.87±171.49[a]
Diabetic + CIAE (500 mg/kg)	341.7±48.91[a]	79.30±4.01[a]	2027.19±185.53[a]
Diabetic + PFAE (250 mg/kg)	360.2±34.27[a]	81.48±3.87[a]	1994.21±194.34[a]
Diabetic + PFAE (500 mg/kg)	368.4±29.06[a]	83.55±3.21[a]	2097.71±49.37[a]

[Ψ]*Values are mean±SD of seven rats in each group (n=7).* [a]*Significant difference was detected in experimental groups comparing with normal group at P<0.05.* [b]*Significant difference was detected in experimental groups comparing with diabetic control group at P<0.05*

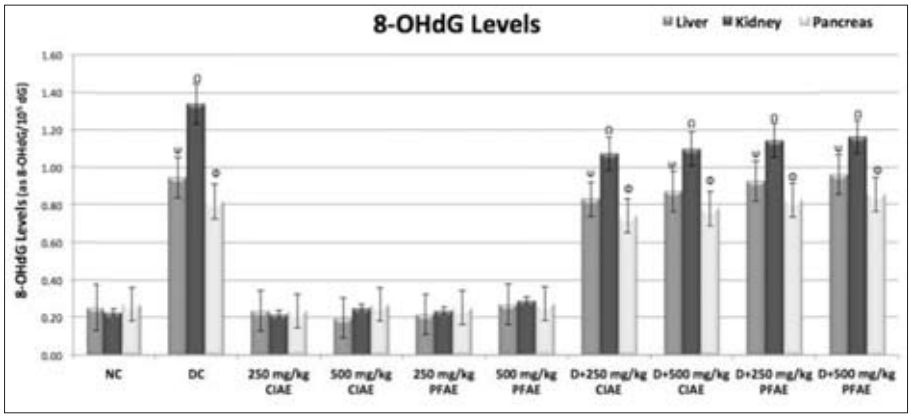


Fig 5. The effect of *C. islandica* (CIAE) and *P. furfuracea* (PFAE) extracts on 8-OHdG levels on liver, kidney and pancreas tissues in normal and diabetic rats (n=7); The symbols used for different tissues indicate the groups that are significantly difference from control group

Şekil 5. *C. islandica* (CIAE) ve *P. furfuracea* (PFAE) ekstraktlarının, normal ve diyabetik sıçanların karaciğer, böbrek ve pankreas dokularında 8-OHdG düzeyleri (n=7); Farklı dokular için kullanılan simgeler, kontrol grubu ile karşılaştırıldığında anlamlı olan grupları temsil eder

pancreas, kidney and liver cells. MDA is an important by-product of lipid peroxidation (LPO), and therefore high levels of MDA lead to oxidative damage to cell membrane lipids [29]. Regrettably, the results presented in this work showed that high glucose allowed the production of MDA effectively in multiple tissues of diabetic rats. It is reported that oxidative stress is a major mediator of tissue and cell injuries [30]. Hence, *in vivo* risk assessment for lichens needs exploration. This study assesses the oxidative effects of *C. islandica* and *P. furfuracea* extracts on pancreas, liver and kidney tissues of rats. Fortunately, MDA levels remained unchanged and lichens alone did not show any adverse effect with respect to increasing dose. Targets of accumulating ROS include proteins involved in antioxidant response. Thus, there is accumulating evidence that interaction of the antioxidant defense system as a regulator of disease development and oxidative stress generation in diabetic patients evokes diabetic organ complications, liver and kidney dysfunctions, and pancreatic β-cell apoptosis [30,31]. These organs contain a number of endogenous antioxidants, to restrict steady state ROS levels. The balance between ROS generation and their elimination by endogenous antioxidant mechanisms play a critical role in preserving organ functions; inappropriate levels of ROS likely precipitate impairment of functions and abnormalities in organ structure [32]. A variety of natural antioxidants exists to scavenge oxygen free radicals and prevents oxidative damage to biological structures. The natural products have antioxidant potential to protect the essential organs against the oxidative damage induced by diabetes [33]. Nowadays, lichens as a novel bioresource for natural antioxidant have been discovered [34]. *P. furfuracea* is shown to modulate the adverse effects of AFB(1) in human blood cells [19]. The antioxidant properties of *C. islandica* and *P. furfuracea* are explained *in vitro* [5,6,19], but not *in vivo*. Our results corroborate this antioxidant activity of lichens and extracts have attractive properties with increasing TAC levels in pancreas, liver and kidney tissues *in vivo*. In these tissues, *C. islandica* significantly increased TAC level and P. furfuracea presented moderate antioxidant supplement for tissues. TAC combines the activities of non-enzymes such as glutathione (GSH) and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) [35,36]. The most abundant intracellular antioxidant, GSH, lowers endogenous ROS and/or exogenous oxidative damage in animal cells GSH-Px enzyme metabolises peroxides such as H_2O_2 and protects cell membranes from LPO [37]. According to Ralph

et al., amino acids may protect against toxicity by serving as precursors for GSH [38]. GSH becomes depleted if cells are deprived of energy substrates. In a hepatopathy model, the effects of *C. islandica* extract are carried out on rats, treated intraperitoneally. It is reported that *C. islandica* extract has hepatoprotective and immuno-stimulating effects [39,40]. *C. islandica* determined adaptogenic-antistress effects, confirmes by its actions on oxidative stress parameters [40]. In this study, *C. islandica* and *P. furfuracea* might be a promising treatment in rats with T1D. Thus, lichen-based therapy is firstly used by this investigastion in treatment of T1D. The results of this study clearly demonstrated that STZ caused significant increases MDA levels in multiple tissues. Conversely, TAC levels in T1D rats were much lower than those healthy controls ($P<0.05$). It was crucial that our research focus on the high antioxidant ability of *C. islandica* for adequate treatment of tissues. After supplementation with *C. islandica* in diabetic animals, decreased in MDA levels and increased in TAC levels were found but the *in vivo* activation of antioxidant capacity by this lichen produced a limited and slight prevention of the tissue cell destructions. The antioxidant status after the *P. furfuracea* treatment was measured to evaluate the effectiveness of a therapy and unfortunately was not improved the clinical response. Again, it was established that there was not any an association between increasing lichen doses and above parameters. As a matter of fact, the high dose of lichens did not allow for a better evaluation of therapeutic effect because of their unchanged antioxidant properties.

The anti-diabetic extracts induce important metabolic changes in T1D [41]. Carbohydrate metabolism disorders in the form of T1D connect with an process connected with the increase of the insulin [42]. And the strict control of the blood glucose level is considered to be essential in order to delay and/or prevent the development of diabetic complications [42]. In this study, we investigated the responses elicited by lichens and how these responses may regulate T1D development. The experimental data showed that *P. furfuracea* lichen did not positively affected plasma insulin level and *C. islandica* extract (250-500 mg/kg) did not provide a significant advantage for T1D rats. Therefore, increase in blood glucose level unfortunately was not inhibited after administration of *C. islandica*. On the other hand, the most significant markers of T1D in both human and animal models are polydypsia and polyphagia [43]. As compared with the normal rats, we determined that the feed and water intake of the diabetic rats were significantly increased. Additionally, the insulin-induced the weight loss was observed after induction of STZ into the animals in present investigation. Animals treated with STZ exhibite continuous hyperglycemia, which coincided with a nearly complete loss of islet β-cells [44,45] Two explanations for weight loss may be presented; firstly, due to polyuria and dehydration the body weight may be declined and, secondly, as the blood glucose level is high there is possibility of muscle breakdown in hyperglycemic rats [46]. Only, the treatment with the *C. islandica* enhanced pancreas function by improving glucose tolerance and increasing β-cell insulin reserve in rats. However, *C. islandica* seemed inadequate to prevent body weight loss in diabetic rats.

In current study, diabetic rats presented renal damages that were evidenced by the elevation in serum urea, uric acid, and creatinine levels, which were considered as significant markers of renal dysfunction [47]. Further our findings, it has been found that the liver was necrotized in diabetic rats due to the deficiency of insulin because of pancreas dysfunction. It is reported that insulin suppress the gene encoding gluconeogenic enzymes [48], ALT is a gluconeogenic enzyme and it is an indicator of impaired insulin signaling [49]. In present study, the activity of AST also increased in diabetic rats, this could be due to an increased release oxidative insult of diabetes lead to damage in hepatocytes [50]. Again, the LDH in serum as a biological marker for liver damage increases [51]. Cell necrosis leads to a rise in the concentration of the LDH enzyme in serum and tissue. The LDH released into the medium provides an index of cell death and membrane permeability to LDH, and an increase in LDH activity in the medium occurs as a result of cell membrane disintegration and enzyme leakage [52]. Thus, the increased activities of ALT, AST and LDH in serum is mainly due to the leakage of these enzymes largely from the liver cytosol into the blood stream [53], which gives an indication of the abnormal function of liver. This study also revealed that hepatocytes retained their capacity to normal function after lichen alone exposures. Moreover, *C. islandica* against T1D presented useful effects on the activities of enzymes without undepending on dose (ALT and AST except for LDH).

The renal impairment, hepatic and pancreatic damage in T1D may be associated with a number of genetic disorders [54]. The method used in the present study is 8-OHdG, which is known to be a sensitive marker of oxidative DNA damage and of the total systemic oxidative stress *in vivo* [55]. 8-OHdG is a product of oxidative DNA damage following specific enzymatic cleavage after the ROS-induced 8-hydroxylation of guanine bases in DNA [56]. Importantly, 8-OHdG appears to play a role in tissue cell injury via the induction of apoptotic cell death [57]. Levels of 8-OHdG have been found to be increased in the pancreatic β cells of STZ-diabetic rats [58,59]. Similarly, increased number of 8-OHdG–positive islet cells was found in our study. In addition, our data confirmed a previous report of the accumulation of 8-OHdG in the nuclear DNA in kidney and liver of diabetic rats. 8-OHdG levels are rapidly normalized by insulin treatment, suggesting the involvement of hyperglycemia in oxidative DNA damage [57-59]. In this study, we provided evidence that lichen extracts alone did not show the production of 8-OHdG during exposure. We also demonstrated that 8-OHdG accumulation in multiple tissues was reduced by the *C. islandica* on the association

between the glycaemic control and levels of 8-OHdG. This is in agreement with a recent study showing that treatment of rat tissues with antioxidants reverse glucose-mediated ROS production [60,61]. Thus, the measurement of 8-OHdG has been associated with potential antioxidant capacity of *C. islandica*.

To our knowledge, this report provides the first account of the *C. islandica* and *P. furfuracea* extracts in vivo. The increasing supplement of *C. islandica* and *P. furfuracea* alone do not show any oxidative and genotoxic effect in pancreas, kidney and liver of diabetic animals. Our opinion: these lichen species are a safe and findings also offer the possibility to early intervene in risk reduction of T1D by using *C. islandica*. As amatter of fact, *C. islandica* improve antioxidant defense system and presents anti-genotoxic effects but the power of *C. islandica* is limited because of intensive oxidative stress in multiple organs of T1D rats.

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