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Original research paper

Antioxidant and antihyperglycemic activities of *Scorzonera cinerea* radical leaves in streptozocin-induced diabetic rats

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Scorzonera species are used for treating various diseases. They are consumed raw, especially in the spring, and have nutritious and dietetic values. This study evaluated the antidiabetic and antioxidant effects of ethanolic extracts of *Scorzonera cinerea* (Sc) radical leaves in diabetes mellitus. Five random groups of Wistar rats (n = 8) were created – control, diabetic, acarbose, Sc-Dried, and Sc-Frozen. Phenolic profiles of extracts were determined by HPLC. Free radical scavenging capacity was measured using DPPH and ABTS tests. The inhibitory effects of Sc extracts on α-glucosidase and α-amylase activities were also evaluated. Moreover, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities, glutathione (GSH) concentration, malondialdehyde (MDA), total antioxidant status (TAS) and total oxidant status (TOS) were analyzed in the liver tissues. While dried *Scorzonera* extract was more effective in α-amylase inhibitory activity, frozen *Scorzonera* extract was more effective in α-glucosidase inhibitory activity. Sc-Dried and Sc-Frozen extracts lowered blood glucose and HbA_{1c} levels, they also increased insulin. Although liver MDA and TOS were significantly increased in the diabetic group, their values were significantly lower in the Sc-Dried- and Sc-Frozen-treated groups. GSH, TAS, and antioxidant enzyme activities decreased in the diabetic group, but Sc-Dried and Sc-Frozen supplements significantly enhanced liver antioxidant values. In conclusion, *S. cinerea* treatment exerts potential hypoglycemic and antioxidant effects in diabetes. Thus, it can be considered as a candidate dietary supplement for health benefits in diabetes.

Keywords: *Scorzonera cinerea*, radical leaves, ethanolic extract, antidiabetic, antioxidant, α-glucosidase

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Diabetes mellitus is a heterogeneous metabolic syndrome characterized by hyperglycemia, which is caused by decreased insulin secretion and/or decreased response in tissues. Hyperglycemia promotes auto-oxidation of glucose to form free radicals and is associated with the activation of the polyol pathway and non-enzymatic glycosylation,

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leading to diabetic complications. Under normal conditions glucose is metabolized *via* the hexokinase pathway; the presence of hyperglycemia, high glucose levels saturate the hexokinase pathway and glucose is then metabolized by the polyol pathway. The polyol pathway converts glucose into sorbitol (polyol) *via* aldose reductase resulting in the accumulation of sorbitol. As sorbitol is not easily transported across cell membranes this increases cellular osmolarity, ultimately leading to cell damage (1). These mechanisms lead to oxidative stress and inflammation. Enhancing the antioxidant status can help reduce oxidative stress and prevent the activation of these pathways (2). There is a limit after which antioxidant enzymes cannot cope with ROS production. Thus, tissue damage may occur due to the imbalance of antioxidant enzymes. Therefore, an external source of free radical scavengers is often necessary for health protection and disease prevention. However, plant-based nutraceuticals offer exhaustive properties in diabetes mellitus (3). Although various pharmaceuticals have been developed for decreasing hyperglycemia, the use of medicinal plants is considered a complementary treatment for diabetes. Knowledge of folk medicine has been transferred to generations and provides valuable information for the discovery of phytochemicals with therapeutic effects (3).

Scorzonera species are edible wild plants and belong to the Asteraceae family and are mainly distributed in Europe, Asia and Africa. They have nutritive and dietetic value, largely owing to the presence of complex carbohydrates, mineral salts, vitamins, and polyphenolic compounds (4, 5). *Scorzonera* species are used in European, Chinese, Tibetan, Mongolian, Libyan, and Turkish traditional and folk medicine against pulmonary diseases, colds, fever, gastrointestinal disorders, and parasitic diseases, as a galactagogue and appetizer, in hepatic pains, abscess, kidney diseases, rheumatism, and diabetes mellitus (6, 7).

A limited experimental study in the literature on *Scorzonera cinerea* (Sc) exists. This study aims to evaluate folkloric information on the antidiabetic and antioxidant effects of the ethanolic extract from the radical leaves of Sc in an experimental diabetic rat model.

EXPERIMENTAL

Chemical reagents such as gallic acid (purity ≥ 99 %), protocatechuic acid (purity ≥ 97 %), chlorogenic acid (purity ≥ 95 %), caffeic acid (purity ≥ 98 %), *p*-coumaric acid (purity ≥ 98 %), ferulic acid (purity ≥ 99 %), *o*-coumaric acid (purity ≥ 97 %), phloridzin (purity ≥ 99 %), rutin (purity ≥ 94 %), ellagic acid (purity ≥ 95 %), quercetin (purity ≥ 95 %), α-amylase, α-glucosidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were procured from Sigma (USA). Acarbose (tablets Glucobay®, Turkey) was procured from local pharmacy. All other chemicals and reagents used were of analytical grade and also procured from Sigma and Merck (Germany).

Plant material and extraction

S. cinerea was collected before and after flowering from Van, Turkey, in April 2017. The plant was identified, and a specimen after flowering was deposited at the Herbarium of Van Yüzüncü Yıl University, Van, Turkey. Some radical leaves collected before the flowering of the plant were dried outdoors, under a shade, and some were frozen at −22 °C. Frozen samples were first thawed and then processed. The dried and frozen radical leaves of the plant were cut up and extracted with 75 % aqueous ethanol at 50 °C for 3 h by continuous

stirring. The extract was filtered through a Büchner funnel and a 45-μm PTFE syringe filter, centrifuged (Hettich Universal 320r, Germany), and then evaporated under reduced pressure at 40 °C (IKA RV 10, Germany). Results of dry samples were expressed on a dry mass (m_d) basis, whereas the results of frozen samples were expressed on a wet mass basis (m_w).

HPLC analysis of an ethanolic extract of the radical leaves of S. cinerea

HPLC analysis was performed according to the Akkol *et al*. (8) with some modifications. As described previously this HPLC method was developed and validated to analyze phenolic acids and flavonoids. HPLC analysis was conducted on a Thermo-Finnigan Surveyor HPLC system (Thermo Fisher Scientific, USA). Thermo Finnigan Surveyor PDA Plus detector was used for monitoring at 254–280 nm and the peak areas were integrated using the ChromQuest software. Elution was performed on the C18 column (Zorbax extend-C18, 4.6×150 mm, 5 μm) using an isocratic solvent system for 60 min at 35 °C. The mobile phase used was H_2O/ACN/AcOH (75:25:0.5, *V/V/V*). The flow rate was 1 mL min^{-1} and the injection volume was adjusted to 20 μL. Identification of peaks was conducted against external standards dissolved in methanol.

Determination of mineral components in the radical leaves of S. cinerea

The dry-ashing method was used for mineral component analysis (9). The radical leaves of *S. cinerea* were weighed in a porcelain crucible and dried in the oven. Thereafter, the samples were turned to ash by gradually increasing the temperature to 500–550 °C in a muffle furnace for 24 h. The ash was dissolved with 1 mol L^{-1} nitric acid. These solutions were used for elemental analysis using an atomic absorption spectrophotometer (iCE 3000 AAS Series, Thermo Fisher Scientific, USA). The concentration of each element was calculated against standard solutions of known concentrations.

Determination of total phenolic and total flavonoid content

Total phenolic content (TPC) in the Sc extracts was determined by the modified Folin-Ciocalteau reagent method (10), using gallic acid as a standard. TPC was calculated as mg gallic acid equivalent (GAE) per 100 g dry and wet mass, resp., dried and frozen radical leaves of Sc. Total flavonoid content (TFC) was determined by the $AlCl_3$ method (11) using quercetin as a standard. TFC was calculated as mg quercetin equivalent (QE) per 100 g dry and wet mass, for dried and frozen radical leaves of Sc, resp.

DPPH radical scavenging activity

The free radical-scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (12). Briefly, 100 μL diluted plant sample or Trolox standard and 3.90 mL methanolic solution of DPPH$^\bullet$ (6×10^{-5} mol L^{-1}) were mixed in a tube and vortexed. The tubes were incubated for 30 min at room temperature in the dark; thereafter, absorbance was measured against methanol at 517 nm (Boeco-S22 UV-Vis spectrophotometer, Boeco, Germany). DPPH activity was expressed as a half-maximal inhibitory concentration, which was calculated using a graph.

ABTS assay

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (7 mmol L^{-1}) was dissolved in 2.45 mmol L^{-1} potassium persulfate solution (final concentration) prepared in distilled water (13). This stock solution was left to react in the dark at room temperature for 12–16 h to form the ABTS radical cation ($ABTS^{\bullet+}$). The $ABTS^{\bullet+}$ solution was diluted with distilled water to an absorbance of 0.700 ± 0.020 at 734 nm (13). Briefly, 20 μL of diluted plant sample or Trolox standard were added to the 1980 μL of $ABTS^{\bullet+}$ solution in a microcentrifuge tube and vortexed. The tubes were left exactly for 6 min at room temperature in the dark after which the absorbance was measured at 734 nm. Percent inhibition was calculated using a graph and expressed as half-maximal inhibitory concentration.

Determination of α-amylase and α-glucosidase inhibitory activity of S. cinerea

α-amylase and α-glucosidase inhibitory activities of the Sc extracts were determined as described previously (14), with some modifications.

For α-amylase inhibitory activity, 100 μL of α-amylase (2 U mL^{-1}) in 0.02 mol L^{-1} phosphate buffer (pH 6.9) was mixed with 200 μL of various concentrations of the extract and pre-incubated at 37 °C for 10 min. Thereafter, 100 μL of 1 % starch solution (in 0.02 mol L^{-1} phosphate buffer, pH 6.9) were added as a substrate and incubated at 37 °C for 15 min. The reaction was stopped by adding 200 μL of 1 % dinitrosalicylic acid and the mixture was then incubated in boiling water for 10 min, then cooled to room temperature and diluted with 2 mL distilled water. The absorbance was measured at 540 nm. Acarbose was used as a standard. The α-amylase inhibitory activity of the extract was expressed as a half-maximal inhibitory concentration, which was determined graphically.

To determine the α-glucosidase inhibitory activity of the extract, 60 μL of α-glucosidase (1 U mL^{-1}) in phosphate buffer (0.1 mol L^{-1}, pH 6.8) was mixed with 120 μL of various concentrations of the extract and pre-incubated at 37 °C for 10 min. Thereafter, 120 μL of 5 mmol L^{-1} 4-nitrophenyl α-D-glucopyranoside was added as a substrate and incubated at 37 °C for 15 min. The reaction was terminated by adding 300 μL of 0.1 mol L^{-1} Na_2CO_3. The absorbance of released *p*-nitrophenol was read at 405 nm. Acarbose was used as a standard. α-glucosidase inhibitory activity of the extract was expressed as a half-maximal inhibitory concentration, which was determined graphically.

The extraction, total phenolic and total flavonoid content, DPPH and ABTS, α-amylase and α-glucosidase inhibitory activities were performed in triplicate.

Animals

Forty healthy male rats (Wistar albino) weighing 200–300 g and 2–3 months of age were procured from the Experimental Application and Research Center, Van Yüzüncü Yıl University (Turkey). The rats were placed in standard plastic rat cages and housed at 22 ± 2 °C, 50 % humidity, and 12-h day/night cycles. This study was approved by Van Yüzüncü Yıl University Animal Researches Local Ethics Committee and procedures complied with the Guidelines for the Care and Use of Laboratory Animals.

Experimental protocol

The rats were randomly divided into five experimental groups (n = 8). Streptozocin (STZ) was administered at 45 mg kg^{-1} bm, *i.p.* Rats with glucose levels ≥ 200 mg per 100 mL

were considered diabetic 3 days after STZ injection. The experimental groups were: 1 – control group (CG), administered 1 mL citrate buffer *i.p.* only, 2 – diabetic group (DG), injected with a single dose of 45 mg kg^{-1} bm STZ, *i.p.*, 3 – diabetic+Sc-Dried, where diabetic rats were treated with 100 mg kg^{-1} bm dried *Scorzonera* extract, 4 – diabetic+Sc-Frozen group, where diabetic rats were treated with 100 mg kg^{-1} bm frozen *Scorzonera* extract, and 5 – diabetic+acarbose (Ac) group, where diabetic rats were treated with 50 mg kg^{-1} bm acarbose. Both *Scorzonera* extracts and acarbose were administered daily, 3 days after STZ injection, using an intragastric tube.

Before administering acarbose, Glucobay® tablets were triturated in the porcelain mortar and dispersed with physiological saline. Excipients of Glucobay® are microcrystalline cellulose, silica colloidal anhydrous, magnesium stearate and maize starch.

The rats were fed standard chow and tap water *ad libitum* for 21 d. Blood and tissue samples were collected after the rats were anesthetized with ketamine and xylazine at the end of the experiment.

Biochemical analyses

Rat liver tissue was homogenized in ice-cold phosphate-buffered saline (pH 7.4) using a titanium probe homogenizer (Sonopuls HD 2200, Bandelin, Germany) for 3 min and centrifuged at 8570×*g* for 30 min at 4 °C. The supernatants were used to analyze glutathione (GSH) concentration, lipid peroxidation (malondialdehyde, MDA), superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) activity, total antioxidant status (TAS) and total oxidant status (TOS).

Malondialdehyde was measured at 532 nm using the method of Draper and Hadley (15), based on TBA reactivity. GSH concentration was measured at 412 nm according to the method described by Beutler *et al*. (16). SOD activity was assayed using a commercially available kit (Ransod, Randox Laboratories Ltd., UK) by calculating the percentage inhibition of formazan dye at 505 nm (17). GPx activity was determined using a commercially available kit (Ransel, Randox Laboratories) at 340 nm, based on the catalytic oxidation of cumene hydroperoxide to reduced glutathione, as described by Günzler *et al*. (18). The catalase activity was estimated using the Aebi method (19) at 240 nm based on breaking down H_2O_2 and transforming it into water and oxygen. Total antioxidant status and total oxidant status were evaluated using a commercially available kit (Rel Assay Diagnostic, Turkey), as described by Erel (20, 21). The TAS method is based on the conversion of the $ABTS^{\bullet+}$ radical into ABTS by the antioxidants in the sample (20). The TOS method is based on the oxidation of ferrous ion complexes to the ferric form by the oxidants in the sample (21). The oxidative stress index (OSI) is the ratio of TAS and TOS and is used to express the status of oxidative stress in tissues. OSI was calculated according to the following formula:

$$\text{OSI (arbitrary unit)} = (\text{TOS/TAS}) \times 100$$

Serum levels of enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), were evaluated using a commercial kit based on the enzymatic colorimetric method (Roche Modular autoanalyzer, Roche Diagnostics, Switzerland). Insulin levels were measured using the electrochemiluminescence immunoassay (ECLIA) (Architect İ4000SR, Abbott

Table I. Amount of phenolic compounds of dried and frozen ethanolic Scorzonera cinerea *radical leaves extracts (mg kg^{-1})*

	GA	PCA	ChA	CA	*p*-Cou	FA	*o*-Cou	Phz	Ru	EA	Qe	TPC[a]	TFC[b]
Sc-Dried (m_d)	2286.30	512.10	6560.60	33.90	267.40	21.30	106.50	Nd	779.50	nd	5.80	57 ± 1	110 ± 6
Sc-Frozen (m_w)	154.20	124.30	36.70	80.60	90.80	17.50	22.30	110.6	105.10	514.40	nd	6.6 ± 0.1 (m_w) 28 ± 1 (m_d)	9.4 ± 0.6 (m_w) 39 ± 3 (m_d)

CA – caffeic acid, ChA – chlorogenic acid, EA – ellagic acid, FA – ferulic acid, GA – gallic acid, *o*-CouA – *o*-coumaric acid, PCA – protocatechuic acid, Phz – phloridzin, *p*-CouA – *p*-coumaric acid, Qe – quercetin, Ru – rutin

Sc-Dried – dried *Scorzonera cinerea*, Sc-Frozen – frozen *Scorzonera cinerea*, nd – not detected; m_d – dry mass, m_w – wet mass

[a] Gallic acid equivalent (mg g^{-1}), [b] quercetin equivalent (mg g^{-1}).

Laboratories Inc., USA). Glycated hemoglobin (HbA_{1c}) level was determined using an automatic glycohemoglobin analyzer based on HPLC (ADAMS A1c HA-8180T, Arkray Inc., Japan).

Statistical analyses

Data were expressed as mean and standard deviation. One-way analysis of variance was used to determine significant differences between groups followed by Tukey's HSD test. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

This study aims to determine the antidiabetic and antioxidant effects of ethanolic extract of *Scorzonera cinerea* radical leaves on STZ-induced diabetic rats. In addition to the traditional drying process, it is aimed to investigate the effect of the freezing process.

Total phenolic and total flavonoid content

The amount of phenolic compounds in dried and frozen leaves of *S. cinerea* ethanolic extract is shown in Table I and HPLC chromatograms in Fig. 1. While chlorogenic acid and gallic acid are the main compounds in the dried radical leaves of *Scorzonera cinerea*, ellagic acid is the main component in the frozen radical leaves. These results together with the previous studies confirm that the main component in dried *Scorzonera cinerea* is chlorogenic acid (8, 22). Although phloridzin and ellagic acid could not be detected in the dry sample, they were found in the frozen sample. However, while quercetin was also found in the dry sample, it was not detected in the frozen sample. TPC and TFC were supported by the HPLC results (Table I). The Sc-Dried extract was a better source of total phenolics with 57 ± 1 mg GAE g^{-1} (m_d) than Sc-Frozen extract with 28.1 ± 0.1 mg GAE g^{-1} (m_d), namely, 6.6 ± 0.1 mg GAE g^{-1} (m_w). However, the TFC were 110 ± 6 mg QE g^{-1} for Sc-Dried ethanolic extract (m_d) against 39 ± 3 mg QE g^{-1} of Sc-Frozen ethanolic extract (m_d), *i.e.*, 9.4 ± 0.6 mg QE g^{-1} (m_w). A recent study conducted on methanolic *S. tomentosa* extracts measured 40.33 mg GAE g^{-1} for TPC (23).

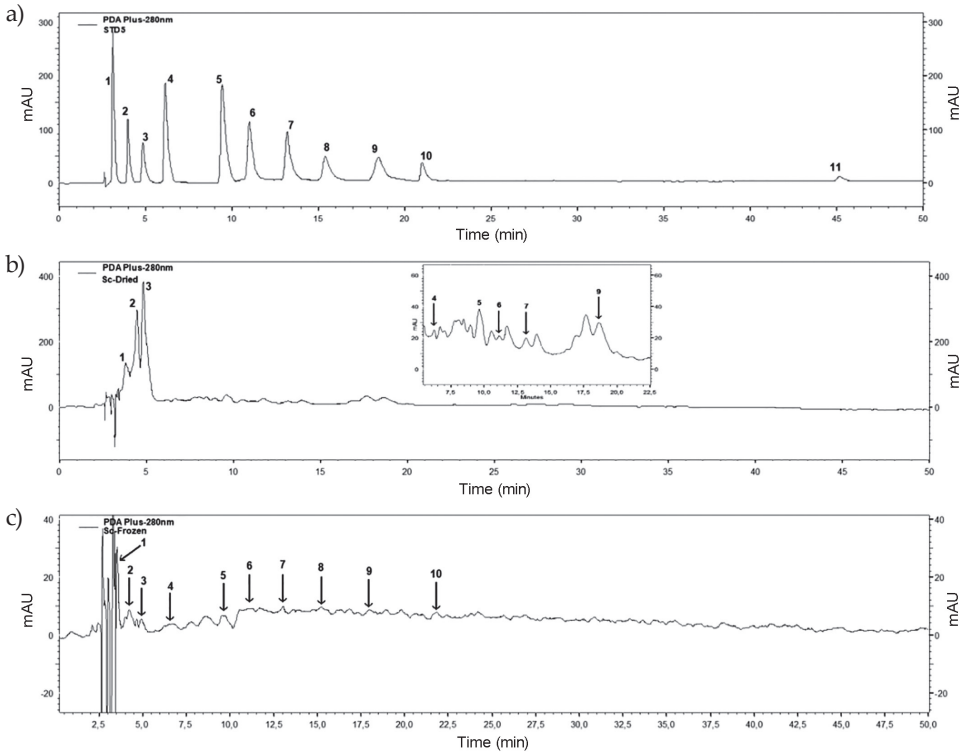


Fig. 1. Chromatograms of: a) standard mixture in methanol, b) dried *Scorzonera cinerea* ethanolic extract, and c) frozen *Scorzonera cinerea* ethanolic extract. Key to the peaks: 1 – gallic acid, 2 – protocatechuic acid, 3 – chlorogenic acid, 4 – caffeic acid, 5 – *p*-coumaric acid, 6 – ferulic acid, 7 – *o*-coumaric acid, 8 – phloridzin, 9 – rutin, 10 – ellagic acid, 11 – quercetin.

Elemental composition of S. cinerea *radical leaves*

The mineral components of the leaves of *S. cinerea* are summarized to indicate the nutritive and dietetic value (Table II). It is rich in potassium, calcium, and magnesium. Considering the dietary reference intake of minerals for adult males [report by Institute of Medicine (24)], *S. cinerea* radical leaves (100 g dry mass) can supply 133 % of Ca, 228 % of Fe, 136 % of Mg, 41 % of K, 6 % of Na, 260 % of Mn, 30 % of Zn, and 153 % of Cu daily requirement. Especially, Cu, Zn, and Mn are important for the activity of SOD which provides a first-line defense of the enzymatic antioxidant system against oxidative damage from superoxide radicals.

DPPH and ABTS radical scavenging activity

The half-maximal inhibitory concentration values of DPPH and ABTS of dried *S. cinerea* were found lower when compared to frozen *S. cinerea* (Table III). The Sc-Dried extract

Table II. Mineral components of Scorzonera cinerea *radical leaves (mg per 100 g dry mass)*

	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Scorzonera cinerea radical leaves	1938.23	1332.56	543.83	134.66	18.30	6.08	3.28	1.38

Table III. Half-maximal inhibitory values for DPPH and ABTS of ethanolic Scorzonera cinerea *radical leaves extracts*

Sample	DPPH (mmol L^{-1} TE g^{-1} extract)	ABTS (mmol L^{-1} TE g^{-1} extract)	DPPH (mg mL^{-1})	ABTS (mg mL^{-1})
Sc-Dried[a]	0.459 ± 0.003	0.068 ± 0.002	4.748 ± 0.027	0.999 ± 0.077
Sc-Frozen[b]	4.864 ± 0.168	0.854 ± 0.041	49.284 ± 3.492	11.243 ± 0.733
Trolox (mmol L^{-1})	0.604 ± 0.011	0.782 ± 0.080		

Sc-Dried – dried *Scorzonera cinerea*, Sc-Frozen – frozen *Scorzonera cinerea*, TE – Trolox equivalent,
[a] Dry mass basis, [b] wet mass basis
Mean ± SD, $n = 3$.

has shown remarkable free radical scavenging ability as compared to Sc-Frozen extract. Various phenolic compounds could be affected differently from the stress caused by drying or freezing. This effect may be attributed to the polyphenol oxidase (PPO) activity. It is during the thawing process that vacuolar membranes become disorganized allowing for the decompartmentalisation of PPO and its substrates resulting in polyphenol oxidation into quinones (25). Also, during the freezing process, PPO maintains its activity for some time under low-temperature conditions, such as −18 °C (26). Herein, Sc extracts exhibited DPPH and ABTS scavenging activity in proportion to their total phenolic and flavonoid content. Similar to the current study of dried *S. cinerea* are the results of Milella *et al*. (5) reporting on the dried *S. papposa* with TPC of 54.5 ± 3.5 mg GAE g^{-1}.

Table IV. α-amylase and α-glucosidase half-maximal inhibitory values of ethanolic Scorzonera cinerea *radical leaves extracts*

Sample	α-amylase	α-glucosidase
Sc-Dried (mg mL^{-1})[a]	0.037 ± 0.000	0.074 ± 0.002
Sc-Frozen (mg mL^{-1})[b]	0.053 ± 0.005	0.067 ± 0.000
Acarbose (mg mL^{-1})	0.380 ± 0.019	0.420 ± 0.010
Acarbose (mmol L^{-1})	0.592 ± 0.028	0.653 ± 0.015

Sc-Dried – dried *Scorzonera cinerea*, Sc-Frozen – frozen *Scorzonera cinerea*; [a] Dry mass basis, [b] wet mass basis
Mean ± SD, $n = 3$.

α-amylase and α-glucosidase inhibitory activity

In this study, α-glucosidase half-maximal inhibitory values of dried and frozen *Scorzonera* extracts are 0.074 and 0.067 mg mL^{-1}, resp. (Table IV). For acarbose, this value was 0.420 ± 0.01 mg mL^{-1}. Kongstad *et al.* (27) reported that the α-glucosidase inhibitory activity of methanolic and ethyl acetate extracts of *S. suberosa* leaves had a half-maximal inhibitory values of 115.1 and 24.8 mg mL^{-1}, resp., and the IC_{50} value of acarbose was 0.47 mg mL^{-1}, similar to our findings.

Hypoglycemic activity

Both Sc extracts are effective in reducing blood glucose level and HbA_{1c} and in increasing plasma insulin ($p < 0.05$) (Table V). The weekly blood glucose of the rats is represented in Fig. 2. Glucose decreased from 406 ± 70 in the diabetic group to 289 ± 42 in the Sc-Frozen group and 242 ± 20 mg per 100 mL in the Sc-Dried group, compared to 179 ± 26 mg per 100 mL in the control group. Moreover, the Sc-Dried and Sc-Frozen groups exhibited a more efficient reduction in blood glucose levels than the acarbose group (334 ± 49 mg per 100 mL). Blood glucose took to decrease in all treatment groups from the first week to the 3rd week. However, among these weeks, it is observed that the highest decline is in the Sc-Dried group (Fig. 2). Besides, HbA_{1c} significantly decreased in Sc-Dried (6.3 ± 0.3 %) and Sc-Frozen (6.5 ± 0.4 %) administered groups compared to diabetic group (8.1 ± 0.4 %). With the help of extracts treatment glucose levels decreased, the percentage of HbA_{1c} also correspondingly decreased. Recently, Donia (4) administered 200 and 400 mg kg^{-1} *S. alexandrina* extract orally to healthy rats for 35 days consecutively and found that both doses were effective in reducing blood glucose. In the previous investigation, 15 d of chlorogenic acid treatment under diabetic conditions attenuated blood glucose levels (28). The presence of ChA as the main compound

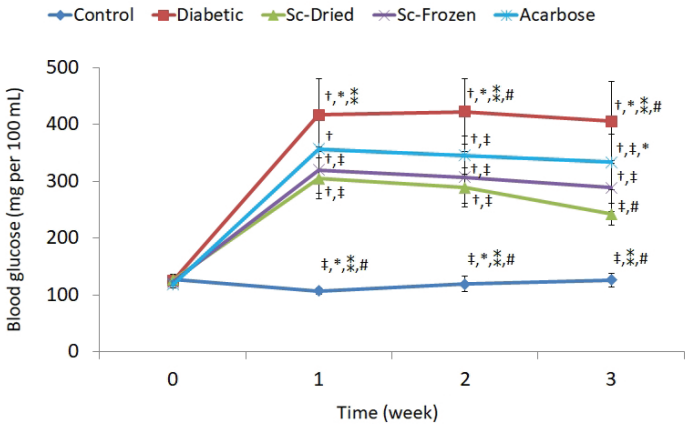


Fig. 2. Weekly blood glucose levels of control and diabetic rats (mean ± SD, $n = 8$). Sc-Dried – dried *Scorzonera cinerea* administered group, Sc-Frozen – frozen *Scorzonera cinerea* administered group, Ac – acarbose administered group. Significant difference at $p < 0.05$ *vs.*: † control group, ‡ diabetic group, * Sc-Dried group, ⁑ Sc-Frozen group, # acarbose group.

in the dried radical leaves of *S. cinerea* may be partly responsible for its anti-diabetic effect. Conversely, the most abundant compounds in frozen *S. cinerea* are ellagic acid, gallic acid, protocatechuic acid and phloridzin (phlorizin). Phloridzin is a competitive inhibitor of the sodium-glucose co-transporter type 1 and 2 (SGLT1 and SGLT2) in the small intestine (29). By blocking SGLT1, glucose absorption from the small intestine is reduced, and by blocking SGLT2, re-absorption of glucose in the kidney is reduced and its excretion is increased (30). Moreover, the synergistic effects of phenolic compounds in *S. cinerea* may contribute to the hypoglycemic effect. Plasma insulin levels increased in the groups treated with Sc-Dried (0.82 ± 0.1 μg mL^{-1}) and Sc-Frozen (0.58 ± 0.1 μg mL^{-1}) extracts for 270 and 160 %, respectively, compared to that in diabetic rats (0.22 ± 0.04 μg mL^{-1}). This may be due to the insulinotropic effect of phytochemicals in *Scorzonera*. Meng *et al*. (31) reported that ChA exerts its hypoglycemic effect by stimulating glucose uptake in both insulin-sensitive and insulin-resistant adipocytes. Besides, ChA increases the stimulation of insulin secretion from rat islets (31). Gallic acid can also increase plasma insulin levels (32). Moreover, the genus *Scorzonera* is known to accumulate inulin-type carbohydrates (33, 34) that are a kind of fermentable fructans that can reduce glucose and ameliorate insulin resistance (35).

Liver function biomarkers

Although serum enzymes significantly increased in the diabetic animals, the administration of Sc-Dried and Sc-Frozen extracts resulted in the recovery of serum enzymes and assisted to decrease these values ($p < 0.05$) (Table V). It is found that STZ-mediated diabetic liver injury elevated TNF-α-related liver serum enzymes (36). However, it has been noted that *S. cinerea* and *S. latifolia*, *S. tomentosa*, *S. mollis*, and *S. eriophora* can inhibit TNF-α; herbal cures can improve liver serum enzymes by inhibition or suppression of TNF-α due to antioxidant properties (22). *S. alexandrina* extract shows hepatoprotective activity in CCl_4-induced liver damage in rats by reducing ALT and AST (4). However, Zhang *et al*. (37) reported that AST, ALT, LDH, and MDA levels are significantly lower in the group treated with *S. austriaca* than those in the CCl_4-intoxicated group (37).

Table V. Serum parameters in experimental groups of rats

Serum	1 – Control	2 – Diabetic	3 – Sc-Dried	4 – Sc-Frozen	5 – Ac
Glucose (mg per 100 mL)	$179 \pm 26^{\ddagger,\text{⁂},\#}$	$406 \pm 70^{\dagger,*,\text{⁂},\#}$	$242 \pm 20^{\ddagger,\#}$	$289 \pm 42^{\dagger,\ddagger}$	$334 \pm 49^{\dagger,\ddagger,*}$
HbA_{1c} (%)	$3.9 \pm 0.1^{\ddagger,*,\text{⁂},\#}$	$8.1 \pm 0.4^{\dagger,*,\text{⁂},\#}$	$6.3 \pm 0.3^{\dagger,\ddagger,\#}$	$6.5 \pm 0.4^{\dagger,\ddagger}$	$6.8 \pm 0.3^{\dagger,\ddagger,*}$
Insulin (μg mL^{-1})	$1.09 \pm 0.09^{\ddagger,*,\text{⁂},\#}$	$0.22 \pm 0.04^{\dagger,*,\text{⁂},\#}$	$0.82 \pm 0.10^{\dagger,\ddagger,\text{⁂},\#}$	$0.58 \pm 0.10^{\dagger,\ddagger,*,\#}$	$0.35 \pm 0.06^{\dagger,\ddagger,*,\text{⁂}}$
AST (U L^{-1})	$93 \pm 13^{\ddagger,*,\text{⁂},\#}$	$517 \pm 58^{\dagger,*,\text{⁂},\#}$	$362 \pm 40^{\dagger,\ddagger}$	$348 \pm 40^{\dagger,\ddagger}$	$432 \pm 66^{\dagger,\ddagger}$
ALT (U L^{-1})	$32 \pm 3^{\ddagger,\#}$	$50 \pm 6^{\dagger,*,\text{⁂}}$	$38 \pm 5^{\ddagger}$	$39 \pm 5^{\ddagger}$	$44 \pm 6^{\dagger}$
LDH (U L^{-1})	$950 \pm 149^{\ddagger}$	$1321 \pm 171^{\dagger,*,\text{⁂},\#}$	$1011 \pm 190^{\ddagger}$	$1059 \pm 143^{\ddagger}$	$1055 \pm 150^{\ddagger}$
ALP (U L^{-1})	$248 \pm 37^{\ddagger,*,\text{⁂},\#}$	$905 \pm 89^{\dagger,\text{⁂},\#}$	$565 \pm 101^{\dagger,\ddagger,\#}$	$654 \pm 70^{\dagger,\ddagger}$	$717 \pm 94^{\dagger,\ddagger,*}$

Sc-Dried – dried *Scorzonera cinerea* administered group, Sc-Frozen – frozen *Scorzonera cinerea* administered group, Ac – acarbose administered group; Mean ± SD, $n = 8$; Significant difference at $p < 0.05$ *vs.*: † control group, ‡ diabetic group, * Sc-Dried group, $^{\text{⁂}}$ Sc-Frozen group, $^{\#}$ acarbose group.

Oxidative stress and antioxidant activity

In the current study, liver MDA and TOS (Figs. 3a,b) were significantly elevated in the diabetic group in comparison with the control group ($p < 0.05$). MDA increased from the control group value of 125 ± 14 nmol mg^{-1} tissue to 186 ± 21 nmol mg^{-1} tissue in the diabetic group. However, Sc-Dried and Sc-Frozen treatment reduced MDA back to 99 ± 13 and 108 ± 10 nmol mg^{-1} tissue, resp. Besides, even acarbose administration caused an increase in MDA (200 ± 23 nmol mg^{-1} tissue). TOS, another indicator of oxidative stress, increased in the diabetic group compared to the control group, 132 ± 19 *vs.* 101 ± 11 μmol H_2O_2 equiv. L^{-1}. On the other hand, TOS value was found significantly lower in the Sc-Dried (91 ± 6 μmol H_2O_2 equiv. L^{-1}) and Sc-Frozen (100 ± 8 μmol H_2O_2 equiv. L^{-1}) administered groups compared with the diabetic group. On the other hand, TOS was 119 ± 14 μmol H_2O_2 equiv. L^{-1} in the acarbose group.

Oxidative status increases in diabetic conditions and leads to tissue damage (2). Moreover, GSH concentration and SOD, GPx, and CAT activities of the liver were significantly decreased in diabetic rats compared to control group rats, indicating suppressed liver antioxidant defense against reactive oxygen species (ROS). GSH was significantly decreased (2.27 ± 0.4 *vs.* 6.63 ± 0.5 nmol mg^{-1} tissue) in the diabetic group compared to the control group. Recently, diabetics were shown to have glutathione deficiency and reduced antioxidant

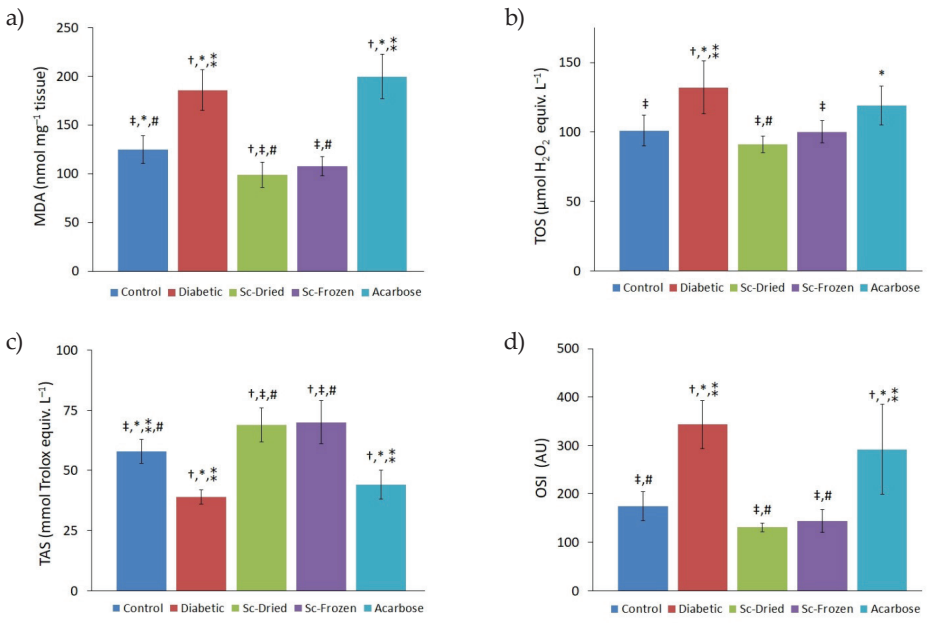


Fig. 3. a) MDA levels, b) TOS, c) OSI, d) TAS of control and diabetic rats (mean ± SD, $n = 8$).

Sc-Dried – dried *Scorzonera cinerea* administered group, Sc-Frozen – frozen *Scorzonera cinerea* administered group, Ac – acarbose administered group. Significant difference at $p < 0.05$ *vs.*: † control group, ‡ diabetic group, * Sc-Dried group, ⁑ Sc-Frozen group, # acarbose group.

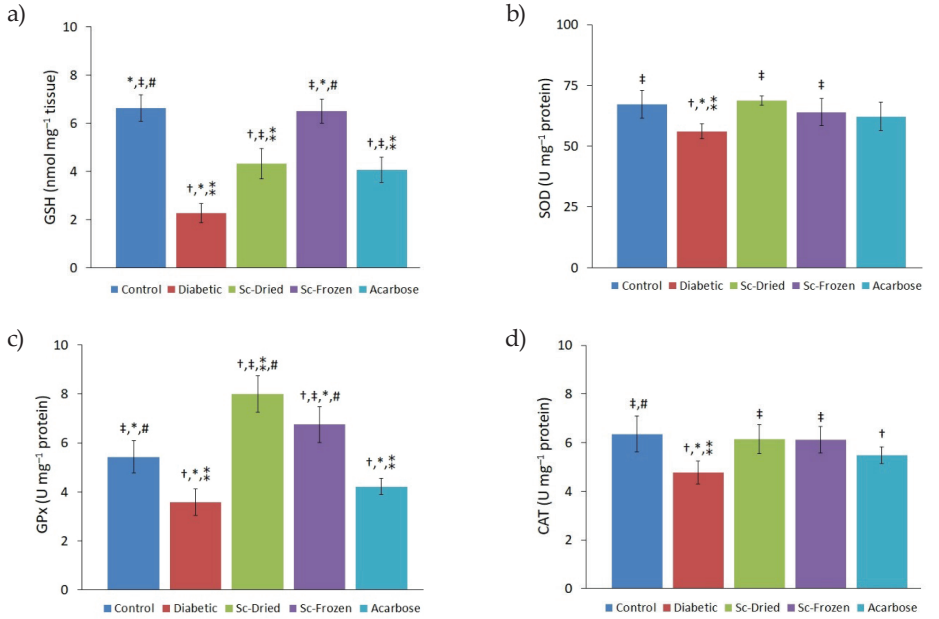


Fig. 4. a) GSH concentration, b) SOD, c) GPx, d) CAT (D) activities of control and diabetic rats (mean ± SD, n = 8). Sc-Dried – dried *Scorzonera cinerea* administered group, Sc-Frozen – frozen *Scorzonera cinerea* administered group, Ac – acarbose administered group. Significant difference at $p < 0.05$ *vs.*: † control group, ‡ diabetic group, * Sc-Dried group, ⁑ Sc-Frozen group, # acarbose group.

capacity (38), which is in accordance with this study. Due to increased blood glucose levels, competition for NADPH between aldose reductase in the polyol pathway and GSH-reductase may occur (39). This results in GSH deficiency; consequently, this may cause other antioxidant enzymes, such as GPx, which uses GSH as a substrate, to decrease. However, Sc-Dried and Sc-Frozen administration increased the GSH level to 4.32 ± 0.6 and 6.50 ± 0.5 nmol mg^{-1} tissue, resp. Thus, Sc-Dried and Sc-Frozen co-treatment can significantly recuperate liver antioxidant capacity. Overall, the administration of Sc-Dried and Sc-Frozen contributed to an increase in TAS and alleviation of TOS in liver tissue. SOD, GPx and CAT activities were lowest in the diabetic group 56 ± 3, 3.6 ± 0.5, and 4.8 ± 0.5 U mg^{-1} protein, resp. However, Sc-Dried administration increased these antioxidant activities to 69 ± 2, 8.0 ± 0.7, and 6.2 ± 0.6 U mg^{-1} protein, resp., whereas Sc-Frozen treatment showed similar values for SOD, GPx and CAT of 64 ± 5, 6.8 ± 0.7, and 6.1 ± 0.5 U mg^{-1} protein, resp. Preventive effects of *S. sinensis* extracts have been reported in the fatty diet by decreasing lipid peroxidation and increasing SOD activity (40). In another study, although the SOD activity significantly decreased in the CCl_4-group, *S. austriaca* treatment increased the SOD activity (37). The antioxidant capacity values are in good agreement with the data for genus *Scorzonera*. Polyphenolic compounds and minerals (such as Cu, Zn, and Mn) found in *Scorzonera cinerea* can provide protection against oxidative stress caused by hyperglycemia by strengthening the enzymatic and non-enzymatic antioxidant defense system.

CONCLUSIONS

The findings reveal that phytochemicals in *S. cinerea* may show liver protective effect by suppressing oxidative stress and boost antioxidant capacity. The study suggests that the phytochemical components in *S. cinerea* mediate its antidiabetic effect by increasing insulin secretion and inhibiting α-glucosidase activity. It is expected that *S. cinerea* may have antidiabetic mechanisms of action by improving insulin sensibility, reducing glucose absorption in the small intestine and increasing the uptake and bioavailability of glucose to the cells. Further studies may focus on these antidiabetic mechanisms of action for *Scorzonera cinerea*.

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