Effects of Scorzonera Cinerea on Immune System and Hematological Parameters in Short-Term Hyperglycemia

Mehmet Ali Temiz

Karamanoğlu Mehmetbey University, Vocational School of Technical Sciences, Programme of Medicinal and Aromatic Plants, Karaman, Türkiye.

Correspondence Author: Mehmet Ali Temiz
E-mail: matemiz@kmu.edu.tr
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ABSTRACT
Objective: Medicinal herbs offer natural remedies for various ailments because of their comprehensive effects. Scorzonera cinerea L. (Sc) is an edible wild plant and is used in traditional medicine against various diseases. The purpose of this study was to investigate the immune system effects of S. cinerea radical leaf extract in diabetic rats.

Methods: The 5 groups were formed — Control, Diabetic, Sc-Dried, Sc-Frozen, and Acarbose. Adenosine deaminase (ADA), xanthine oxidase (XO), and myeloperoxidase (MPO) activities in liver and kidney tissues were analyzed. Hematological parameters were also evaluated.

Results: ADA, XO, and MPO activities in both tissues significantly increased in diabetic conditions. However, Sc treatments significantly decreased liver ADA, liver and kidney XO, and liver and kidney MPO activities. There was no significant change in red blood cell (RBC) parameters. Although platelet (PLT) count and MPV were raised in the diabetic group, these parameters were reduced with Sc-Dried and Sc-Frozen treatment. While lymphocyte (LYM%) significantly decreased in defense cells in the diabetic group, neutrophil (NEU%), monocyte (MO%), and eosinophil (EOS%) elevated. However, a significant decrease was observed in NEU% and EOS% with Sc treatment.

Conclusion: S. cinerea treatment can exert a potential immunoregulatory effect in diabetes. Thus, S. cinerea can be considered as an adjuvant to augment immune system on diabetes.

Keywords: Adenosine deaminase, Hematology, Immunity, myeloperoxidase, Scorzonera cinerea, xanthine oxidase

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic, heterogeneous, and progressive disease that is characterized by hyperglycemia due to deficiency of insulin and/or absence. Diabetes related many diseases that among these are heart, eyes, nerves, and kidney diseases, skin complications, and immune system disorders (1). It has been supported by many researchers that there is also a serious relationship between diabetes and oxidative stress (2,3). Increased free radical production causes the activation of some major pathways such as the polyol pathway, protein kinase C, an increase in the formation of advanced glycation end-products (AGE), and overactivity of the hexosamine pathway. At the same time, these pathways lead to the production of free radicals again and have a role in the pathogenesis of complications (4). Diabetes has significant adverse effects on the immune system. High blood glucose levels increase the activity of immune cells. These cells eventually become depleted and less sensitive; as a result, their effectiveness against invading pathogens is reduced. Protein kinase C is activated by hyperglycemia, thereby inhibiting neutrophil migration, phagocytosis, superoxide production, and microbial killing. Hyperglycemia can also reduce the formation of neutrophil extracellular traps (5). The chronic inflammatory state in diabetics may play a role in impaired immune function, thus increasing susceptibility to infections. On the other hand, regulation of the activities of some enzymes responsible for immunity, such as ADA, MPO, and XO, is important to the proper functioning of the immune system.

Medicinal herbs offer exhaustive properties because of their multiple effects. Natural compounds such as polyphenols have the ability to modulate ADA enzyme activity as well as reverse oxidative damage (6). A fall in ADA levels was greater in diabetic patients taking metformin with garlic than in patients taking only metformin (7). It is known that MPO has a critical role in the onset and progression of acute and chronic inflammatory diseases. Moreover, as the XO pathway is considered an important pathway for the production of reactive oxygen species (ROS) and the high oxidative stress has a significant impact on immunity, XO pathway may have...
a critical role in modulating immunity (8). Studies with plants show promise in reducing inflammation and regulating immunity. A previous study demonstrated that *Withania coagulans* corrects reduced the proinflammatory markers in kidneys (9). Another plant study, *Combretum molle* treatment elicited a decline in MPO and XO activity in diabetic rats (10). Additionally, grape seed extract attenuated both XO and ADA activities in the diabetic rats (11). Previously, *Mesona procumbens* extract downregulated streptozotocin-induced liver XO activity, and it restored renal organic anion transporter 1 and urate transporter expression (12). Recent study, *Rumex crispus* root exhibited potent XO inhibitory activity in *in vitro* assays (13).

The genus *Scorzonera* (Asteraceae) is widely spread in Europe, Asia and Africa. They are edible wild plants and are used up generally raw in spring because of their nutritional and dietary value (3). *Scorzonera* genera are used in traditional medicine in Europe, China, Tibet, Mongolia, Libya, and Turkey to treat gastrointestinal disorders, colds, fever, pulmonary diseases, and parasitic diseases. Moreover, these genera are used as galactagogues and appetizers as well as for rheumatic disorders, renal failure, hepatic pains, abscesses, and diabetes mellitus (3). To the best of our knowledge, there are limited experimental studies on *Scorzonera cinerea* (Sc) in the literature. This study aims to investigate the immune system effects of *S. cinerea* radical leaf extract in diabetic rat.

2. METHODS

2.1. Plant Materials and Extraction

*S. cinerea* was collected from Van, Turkey, in April 2017. The taxonomic identification of the plant was performed and a specimen is kept in the herbarium of Van Yüzüncü Yıl University. Parts of *S. cinerea* radical leaves were dried outdoors approx. 18°C and 55% humidity for 4 days and then powdered. The other parts of them were frozen at ~22°C for 3-month storage. The dried radical leaves were extracted with ethanol (75%) at 50°C for 3 h. The frozen radical leaves were first gradually thawed and then crumbled and extracted with ethanol (75%) at 50°C for 3 h. The extractions were filtered through filter paper and then concentrated under reduced pressure at 40°C (IKA RV3 V, Germany).

2.2. L-Ascorbic Acid Determination

The amount of ascorbic acid was determined by the method of Lee and Coates (14) with some modifications. The plant samples were homogenized with ice-cold metaphosphoric acid (4%) in ice bath. The homogenate was centrifuged at 10000×g for 5 min at 4°C. The upper clear liquid was filtered and immediately injected into the HPLC system. The system consists of a Rhodyne 7725i injector, an LC-20 AD gradient pump, a CTO-10AS VP column furnace, and an SPD-M20A diode array detector. Hypersil Gold aQ C18 column was used with a mobile phase (H₂O:H₂SO₄ 98:2 v/v, pH 2.54). Flow rate of 0.7 mL/min and the injection volume of 20 µL were adjusted at 25°C. The measurement was performed at 244 nm. Ascorbic acid was identified and calculated according to retention times and by comparison with the external standard (y = 86755x + 3611.6, r² = 0.9992).

2.3. Animals

Experiment was performed using 40 healthy *Wistar albino* male rats (200–300 g; 2–3 months of age) which obtained from Van Yüzüncü Yıl University Experimental Application Center. The rats were housed under standard conditions (22±2°C, 50% humidity, and under a 12-h light/dark cycles). They were provided standard chow and tap water *ad libitum*. Ethics Committee of Van Yüzüncü Yıl University approved the study protocol (Date:2019, decision no: 2019/02).

2.4. Experimental Design

Experimental diabetes was induced by a single dose intraperitoneally (i.p) injection of streptozotocin (STZ) (45 mg/kg body weight (bw) in citrate buffer). Rats were allowed to develop diabetes for 3 days. Thereafter, rats with fasting blood glucose levels over than 200 mg/dL were considered diabetic. The rats were divided randomly into 5 groups (n=8) as follows:

- Control group (CG) received single dose of 1 mL citrate buffer i.p and 1 mL physiological saline p.o for 21 days;
- Diabetic group (DG) – diabetic rats who received 1 mL physiological saline orally (p.o) for 21 days after DM induction;
- Sc-Dried group – diabetic rats who were administered 100 mg/kg bw dried Scorzonera extract p.o for 21 days after DM induction;
- Sc-Frozen group – rats who were administered 100 mg/kg bw frozen Scorzonera extract p.o for 21 days after DM induction;
- Acarbose group – rats who were administered 50 mg/kg bw acarbose p.o for 21 days after DM induction. After 21 days, the rats were anesthetized, and then blood and tissue samples were taken.

2.5. Biochemical Analyses

Rat tissues were homogenized with ultrasonic homogenizers in an ice-cold phosphate-buffered solution (pH 7.4) and centrifuged at 8570xg for 30 min at +4°C. The obtained supernatants were used to evaluate ADA, MPO, and XO activities. Protein quantification was measured by modifying the Lowry method (15).

2.6. Measurement of ADA Activity

ADA was measured using the method of Giusti (16). The method is based on the generation of ammonia, which is directly proportional to the extinction of indophenol as a final product. The ammonia reacts with hypochlorite and phenol in an alkaline solution thereby formation of an intense blue color which is measured at 630 nm.
2.7. Measurement of XO Activity
XO was determined using the method of Prajda and Weber (17). The XO method is based on formation of uric acid from xanthine at 37°C. XO activity was measured at 293 nm and calculated in mmol uric acid produced per min.

2.8. Measurement of MPO Activity
MPO was analyzed by the method of Bradley et al. (18). MPO catalyzes the conversion of hydrogen peroxide (H₂O₂) and chloride (Cl⁻) to highly toxic hypochlorous acid (HOCl). The produced oxygen radical (O⁻) reacts with o-dianisidine dihydrochloride to form a colored compound which is measured spectrophotometrically at 460 nm.

2.9. Hematological Parameters
The complete blood count was evaluated using an autoanalyzer (Abaxis Vetscan HM2, Allied analytic, USA).

2.10. Statistical Analyses
All the data were statistically analyzed by one-way ANOVA and Tukey post hoc test. The findings were presented as mean±SD. Value of p< .05 was considered as significantly different.

3. RESULTS
The amount of ascorbic acid in S. cinerea is presented in Table 1. Although ascorbic acid in dried Sc was found, it could not be detected in frozen Sc.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Dried</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid (mg/kg)</td>
<td>36.67</td>
<td>n.d</td>
</tr>
<tr>
<td>n.d, not detected.</td>
<td></td>
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</tbody>
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ADA activities of liver and kidney tissues were increased in the diabetic group compared to the control group (p< .05) (Figure 1). Sc-Dried, Sc-Frozen, and acarbose administration significantly decreased ADA activity in comparison with the diabetic group in the liver. Although kidney ADA activity was low in the Sc groups, it was not significant. Liver and kidney XO activity elevated significantly in the diabetic group than those in the control group (Figure 2). However, XO activity was found to be significantly lower in both tissues in the Sc-Dried, Sc-Frozen, and acarbose administered groups in comparison with the diabetic group. MPO activity in both tissues of the diabetic group was remarkably higher than those in the control group (Figure 3). Notably, Sc-Dried and Sc-Frozen supplementation were decreased MPO activity in the liver and kidney tissues compared to the diabetic group (p< .05). Besides, administration of acarbose also significantly reduced MPO activity in both tissues (p< .05).

The hematological values of the rats are shown in Table 2. There was no significant difference between the groups in RBC and mean corpuscular hemoglobin (MCH) parameters. However, hemoglobin (Hb) was found significantly higher in the Sc-Dried and Sc-Frozen groups than those in the diabetic group. A significant decrease was observed in mean corpuscular volume (MCV) and hematocrit (HCT) in the diabetic group compared to the control. On the other hand, Sc-Dried administration increased HCT and Sc-Frozen administration increased MCV in comparison with the diabetic group. The significant increase in the PLT count and mean platelet volume (MPV) observed in the diabetic group drastically reduced to near normal level following the administration of Sc-Dried and Sc-Frozen. Moreover, acarbose treatment decreased the PLT count in comparison with the diabetic group. As regards to the white blood cell and fragments, while white blood cells (WBC) level was not significantly affected by diabetes, there was a significant decrease in Sc treated groups compared to those in the diabetic group. Whilst the percentage of LYM significantly decreased, NEU%, MO%, and EOS% raised in the diabetic group in comparison with the control group. However, a significant decrease was found in NEU%, MO%, and EOS% in the Sc-Dried group in comparison with the diabetic group. Similarly, the Sc-Frozen group also had a significant decline for NEU% and EOS%.

Figure 1. Liver (A) and Kidney (B) ADA activities of rats.
†: It was significantly different from control group (p< .05), ‡: It was significantly different from Diabetic group (p< .05), *: It was significantly different from Sc-Dried group (p< .05), §: It was significantly different from Sc-Frozen group (p< .05).
Effects of Scorzonera Cinerea on Immune System in Diabetes

4. DISCUSSION

The immune system is affected by many conditions such as smoking and excessive use of alcohol, sedentary lifestyle and irregular sleep, irregular and wrong eating habits or some diseases, one of which is DM (19). Due to the inability to control the spread of invading pathogens in diabetics, hyperglycemia is thought to cause dysfunction of the immune response. For this reason, it is known that diabetics are more susceptible to infections (20). The immune system is a complex and multi-layered system that protects the body against infections and other diseases. So, strengthening the immune system is very important in this sense. Phytochemicals such as polyphenols and vitamins found in medicinal and wild edible plants may have beneficial therapeutic effects on immune system disorders in DM. Polyphenols may provide protection indirectly through the activation of endogenous defense systems and through the modulation of cellular signaling processes such as NF-kB.
activation, glutathione synthesis, MAPK pathway, and PI3/Akt pathway (21). Moreover, polyphenols can enhance the level of IL-21 and decrease the release of IL-1β and IL-6 (22). It is also well known that vitamins especially ascorbic acid is responsible for the immune system.

In the current study, although ascorbic acid in dried Sc was found, it could not be detected in frozen Sc (Table 1). The frozen and thawing processes resulted in the complete loss of ascorbic acid after 3-month storage. It has been stated that thawing at 4°C, which is a common practice, resulted in pigment and ascorbic acid losses (23). This was assumed to result from oxidation by polyphenol oxidases and ascorbate oxidase activities of anthocyanins and ascorbic acid, respectively. Accordingly, it was determined that after thawing, ascorbic acid was almost completely depleted after 48 hours at 20°C (23). Moreover, it has been informed that ascorbic acid continues to degrade during the storage process of frozen products and losses is about 20–50% for fruits and vegetables stored at −18 to −20 °C (24). Since ascorbic acid is a water-soluble vitamin, cellular compartments can be disorganized in the freezing process and can be quickly depleted by ascorbate oxidase in the thawing process (23).

On the other hand, it is normal to have high component concentration in the dry sample, but the ascorbic acid in the wet sample may be below the detectable limit due to the high-water content. Undetected may be due to this. Dried S. cinerea has 36.67 mg/kg of ascorbic acid. Dietary reference intake (DRI) of ascorbic acid is 75 and 90 mg/d for females and males, respectively (25). Therefore, the amount of ascorbic acid in dried S. cinerea can supply the DRI of 40.7–48.9 %.

ADA deaminates adenosine to inosine, and deoxyadenosine to deoxyinosine irreversibly. In the ensuing reaction hypoxanthine is formed. It is regarded as a marker in evaluating cell-mediated immune response (26). It is also suggested that ADA plays an important role in modulating the bioactivity of insulin. In cases of oxidative stress and cell membrane damage, serum ADA is increased (27). ADA activity increased in both tissues of the diabetic group (Figure 1). Current findings are supported by many studies that were found a significant elevation in ADA activity in tissues of STZ-induced diabetic rats (28-30). It has been stated that in type 2 diabetes, ADA activities are mainly raised which has a positive correlation with glycemic parameters (26). In our recent study, it was shown that glycemic parameters such as blood glucose and HbA1c were high in the diabetic group (3). The liver ADA activity significantly decreased with S. cinerea treatment. In studies of various plant extracts, for example, Beta vulgaris leaves and Syzygium cumini seed extracts decreased ADA activity in diabetic conditions through purinergic signaling inhibition (28,29). Bitencourt et al. (29) reported that chlorogenic acid and rutin are the most abundant phenolic compounds in Syzygium cumini. In a previous study, rutin administration reduced the elevated ADA level of STZ-induced diabetic rats (31). S. cinerea contains many phenolic compounds such as chlorogenic acid, gallic acid, ellagic acid, and rutin (3). Therefore, S. cinerea may have been effective in reducing the activity by modulating ADA through the individual phenolics or their synergistic effects.

Hypoxanthine formed ensuing ADA reactions is oxidized to xanthine by XO, and xanthine is also oxidized to uric acid by XO. Namely, an increment in ADA activity causes an increase in XO activity. The reactions catalyzed by XO also produce ROS. The current study demonstrated that diabetes resulted in about 2-fold XO activity than that of control groups in both tissues (Figure 2). The present findings are in line with earlier reports in which diabetes led to ROS generation in tissues through increasing XO activity (32). Fortunately, Sc and acarbose treatments were effective in reducing XO activity. In a previous study, grape seed extract supplementation reduced the plasma XO activity of STZ-induced diabetic rats (11). Many plant extracts and isolated compounds showed XO inhibitory effect such as Citrullus colocynthis leaf extract (33), apigenin and hispidulin isolated from Centaurea virginata aerial parts (34). It was stated that some isolated compounds from Rumex crispus root showed strong XO and α-glucosidase inhibitory effect (13). Recently, it was reported that dried S. cinerea and frozen S. cinerea possess high α-amylase and α-glucosidase inhibitory effects (3). The dried and frozen S. cinerea have α-amylase inhibitory effect 0.037±0.000 and 0.053±0.005 mg/mL, respectively. Besides, α-glucosidase inhibitory effect of dried and frozen S. cinerea are 0.074±0.002 and 0.067±0.000 mg/mL, respectively. However, acarbose has shown α-amylase and α-glucosidase inhibitory effect 0.380±0.019 and 0.420±0.010 mg/mL, respectively (3). ADA and XO activity may have diminished due to both antidiabetic and antioxidant as well as purinergic enzymes inhibitory effects of S. cinerea.

MPO is an activated neutrophil-specific enzyme that regulates modulation of immunological responses and inflammation, inhibition of nitric oxide generation, and modification of lipoprotein function (35). MPO catalyzes the reaction of chloride and H₂O₂ to produce HOCl. MPO also mediates oxidative stress by inducing the generation of ROS and reactive nitrogen species. Therefore, increased stress conditions in diabetes can exacerbate MPO activity. In a previous study compatible with the present study, Gezginci-Oktayoglu et al. found a significant elevation in liver MPO activity in diabetic rats induced with STZ. In the same study, it has been reported that administration of Beta vulgaris leaves extract remarkably restored liver MPO activity in diabetic rats (28). It is estimated that MPO is a marker of an increased inflammatory state and leukocyte count in diabetics. The inflammatory biomarkers such as WBC, NEU%, MO%, and EOS% elevated in the diabetic group (Table 2). Considering that diabetes plays a role in many pathogeneses such as the activation of leukocytes, it can be said that increases in inflammation also contribute to the increase in MPO (36,37). It has been shown that rutin and curcumin pretreatments are effective in preventing hyperlipidemia-induced immune cell activation and inflammation by reducing MPO activities (38). It is known that phenolic compounds alleviate oxidative stress under favour of having antioxidant effects. Therefore,
MPO may be diminished by suppressing stress conditions due to the remarkable phenolic compounds of *S. cinerea*.

Patients with DM have infections more often than those without DM as diabetes negatively influences blood parameters. In the present study, overall significant differences in cellular elements of the blood were observed between the control and diabetic groups (Table 2). Although Hb decreased relatively in the diabetic group, a significant increase was seen in the Sc-treatment groups. More recently, we found that Sc possesses high mineral content (3). Considering the DRI of iron for adult (25), *S. cinerea* can provide with the DRI of 228 % of iron (3). Therefore, Hb may be higher in Sc groups due to high iron content. Although PLT and MPV increased in the diabetic group, Sc administrations effectively reduced these parameters. Acarbose also reduced PLT. However, in previous study, while PLT was not significantly different in diabetics, it has been shown that MPV was independently associated with the presence of diabetes (39). In the current study leukocytes, such as NEU%, MO%, and EOS%, were increased but that of LYM% was decreased in the diabetic group. Diabetes induces apoptosis in lymphocytes, which may explain low LYM% due to impaired immune function in the diabetic states (40). Our findings are consistent with Mahmoud (41), who reported while LYM% decreased in the diabetic group, hesperidin or naringin administration increased LYM%. On the other hand, it can be said that dried Sc provides more support to the immune system due to its vitamin C content and provides a relative relief in defense cells, such as WBC and LYM%. Moreover, dried Sc administration alleviated NEU%, MO%, and EOS%. Similarly, frozen Sc showed a significant decrease for NEU% and EOS%. Mahmoud (41) stated that hesperidin or naringin administration to diabetic rats was effective to reduce NEU% and MO%. Activation of the NF-κB signaling pathway is associated with regulation of inflammatory response. Hyperglycemia is an important mediator of neutrophil dysfunction in DM, as it upregulates the receptor for AGEs on the neutrophil cell surface. AGEs induce oxidative stress and pro-inflammatory gene expression (NF-κB) in multiple cell types, including neutrophils. Activation of NF-κB can be induced in the diabetic state, thereby triggering the initiation of inflammation by neutrophils (42). Anti-inflammatory feature of phenolic compounds is to inhibit neutrophil degranulation, which is a direct way to decrease the release of arachidonic acid by neutrophils and other immune cells. Using an *in vitro* TNF-α and IL-1 production inhibition assay, the anti-inflammatory potential of some Scorzonera extracts, including *S. cinerea*, was confirmed through the inhibition of NF-κB activation (43). Administration of *S. cinerea* may have attenuated the activities of immune cells by regulating the inflammatory response.

**CONCLUSIONS**

Increased ADA, MPO, and XO activities and some hematological parameters may be an important indicator in the immunopathogenesis of diabetes mellitus. This study demonstrated that *S. cinerea* exhibited modulatory effects on immune system. *S. cinerea* can regulate immune function by reducing oxidative stress with its efficient compounds. Naturally, no herb should be preferred to be used as an isolated form of therapy, but it can be used as an adjuvant to regulate immunity.

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**Author Contributions:**

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- Acquisition of data for the study: MAT
- Analysis of data for the study: MAT
- Interpretation of data for the study: MAT
- Drafting the manuscript: MAT
- Raising it critically for important intellectual content: MAT
- Final approval of the version to be published: MAT

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