

Antioxidant Effect of *Carum carvi* on the Immune Status of Streptozotocin - Induced Diabetic Rats Infected with *Staphylococcus aureus*

Gehan Moubarz, Mona Mohamed Taha and Heba Mahdy-Abdallah

Department of Environmental and Occupational Medicine, Environmental Research Division,
National Research Center, Giza, Egypt

Abstract: The rates for both type 1 and type 2 diabetes mellitus have been increasing all over the world. Occupational exposures to organochlorine pesticides, dioxins or metals as arsenic have been associated with increased risk of diabetes. In addition, recent data suggests that toxic substances in the environment, other than infectious agents or exposures that stimulate an immune response, are associated with the occurrence of these diseases. The aim of the present study was to assess the antioxidant role of *Carum carvi* in improving bacterial infection in diabetes. Diabetes was induced in rats by intraperitoneal injection of streptozotocin (STZ). *Staphylococcus aureus* suspension was slowly applied through a wounded area to induce bacterial infection in rats. *Carum carvi* supplementation was started one week later and continued for 6 weeks. Blood was collected through the retro-orbital plexus. Results revealed that *Carum carvi* oil reduced blood glucose in diabetic and diabetic infected rats. In addition results showed an increase in total leucocytic count and total immunoglobulin E and decrease in inflammatory cytokines (interleukin 6, interleukin 1 β and tumor necrosis factor). Thus *Carum carvi* may be used as a co-treatment with conventional anti-diabetic therapy. It may help in improving immune functions, hence attenuating diabetic complications.

Key words: *Carum carvi* • Diabetes Mellitus • *Staphylococcus aureus* • Oxidative Stress • Immune Function

INTRODUCTION

Caraway (*Carum carvi* L., Umbelliferae) is a popular traditional herb used as a spice in food and food products. *Carum carvi* belongs to the family Apiaceae that is one of the earliest cultivated herbs in Asia, Africa and Europe. Caraway seeds contain several components: Carvone, limonene, germacrene D and trans-dihydrocarvone in their oil. Also, their seeds contain trace amounts of other compounds including acetaldehyde, furfural, carveole, pinene, thujone, camphene, phellandrene [1-3].

The antidiabetic effect of caraway products was amply documented. Caraway oil exhibited anti-hyperglycemic activity in streptozotocin (STZ)-induced diabetic rats and increased the body weight [4].

Besides the antimicrobial effect of Caraway seeds, caraway products (oils as well as their aqueous and solvent derived extracts) have shown significant antioxidant activity [2].

Diabetes mellitus (DM) is a metabolic disorder affecting several million individuals. Changes in human behavior and life style have resulted in a dramatic increase in the incidence of diabetes over the world [5]. Moreover the estimated number of diabetic patients would reach 380 million by the year 2025 [6].

DM has been associated with immune dysfunction e.g. reduced T cells response, neutrophil function and humoral immunity disorders [7]. Consequently, DM increases the susceptibility to infections. Oxidative stress also plays an important role in developing complications of DM [8, 9]. Reactive oxygen species (ROS) leads to stimulation of an innate immune response through the induced production of proinflammatory cytokines as TNF-alpha, IL6 and IL-1beta and results in autoimmune diabetes. Treatment of STZ induced diabetic rats with the anti-oxidant butylated hydroxyanisole decreased production of TNF-alpha and IL-1beta by islets of pancreas and peritoneal macrophages [10].

Corresponding Author: Heba Mahdy-Abdallah, Department of Environmental and Occupational Medicine, Environmental Research Division, National Research Center, Postal Code: 12622, Dokki, Giza, Egypt

Foot infections are the most important chronic complications of DM, being one of the most common causes of hospitalization and often resulting in amputation, osteomyelitis and death [11]. Methicillin-resistant *Staphylococcus aureus* is a major pathogen in these infections. The emergence of *S. aureus* strains resistant to multiple antibiotics has made the treatment of infectious diseases uncertain [11, 12]. This has led to the screening of several medicinal plants for their potential antimicrobial and antioxidant activities.

The present study was designed to assess the potential antioxidant and antibacterial effect of *Carum carvi* in STZ-induced diabetic rats.

MATERIALS AND METHODS

The study included thirty adult albino Wistar rats, weighing 170 ± 10 g. Rats were left 2 days for acclimatization at room temperature with a 12 h light/dark cycle before beginning the experimental work. Animals were fed normal rodent chow supplied by (El-Nasr Pharmaceuticals, Chemicals Industries, Egypt). They were allowed free access to drinking water and observed daily. The study protocol had been approved by scientific committees at Cairo University and the National Research Center, Egypt. Permission for animal use and approval of the protocol were obtained from the International Animal Ethics Committee.

Preparation of Streptozotocin for Diabetic Induction: Streptozotocin (STZ) was freshly prepared by dissolving in 0.1M citrate buffer (pH 4.5, Sigma- Aldrich Co., USA) prior to induction.

Preparation of Bacteria for Infection: *S. aureus* strain was kindly provided by Dr/ Lamia Foad from Department of Bacteriology, Faculty of Medicine, Ain Shams University, Egypt. *S. aureus* strain was cultivated on nutrient agar then harvested by centrifugation at $9000 \times g$ for 10 min. The bacteria were suspended in sterile saline solution and centrifuged again. Re-suspension in sterile saline solution and centrifugation were repeated 3 times. The inoculum's suspensions were prepared by re-suspending the washed bacteria in 4 ml of sterile saline solution. The final suspension was adjusted to 10^8 *S. aureus* colony forming unit (CFU).

Preparation of *Carum carvi*: Essential oil of *Carum carvi* was prepared by grinding the seeds (25gm) and the

resulting powder was hydro-distilled for 3 hours. The essential oil was stored in sealed vials under N_2 at $4^\circ C$ until usage.

Methods

Induction of Diabetes: Diabetes was induced in groups II, III, IV and V by injection of freshly prepared STZ solution (50 mg/kg body weight) by intraperitoneal route [13]. Four days after STZ administration, diabetes was confirmed by determination of fasting blood glucose concentration with the help of glucometer. The animals with blood glucose more than 200 mg/dl were selected to compose the diabetic control, diabetic infected control and supplemented groups (groups II to V).

Infection with Bacteria: The backs of diabetic rat were shaved by a razor blade and cut wounds 1 cm in length were produced by surgical blades. One hundred microliters of the bacterial suspension (10^8 CFU *S. aureus* cells) were slowly applied through a micropipette on the wounded area. One week later, the bacterial infection was confirmed by appearance of abscess.

Experimental Design: Rats were divided into 5 groups (each group consisted of 6 rats) as follows:

Group I: Normal control fed with normal rodent chow

Group II: Diabetic control fed with normal rodent chow

Group III: Diabetic infected control fed with normal rodent chow

Group IV: Diabetic rats fed with normal rodent chow and supplemented with *Carum carvi* oil.

Group V: Diabetic infected rats fed with normal rodent chow and supplemented with *Carum carvi* oil.

Treatment with Antioxidant Supplementation: The antioxidant previously prepared, *Carum carvi* oil was fed orally with normal rodent chow at a dose of 50 mg/kg body weight to rats of group IV and V respectively for 6 weeks. Control animals (groups I, II, III) received the same amount of normal rodent chow. At the end of 6 weeks of treatment body weight was recorded.

Blood Collection and Biochemical Analysis: At the end of the experiment, rats were fasted overnight and blood was withdrawn through the retro-orbital plexus using a

glass capillary and collected into EDTA and Non-EDTA dry tubes. The portion of blood on EDTA tubes was used to determine total and differential leukocyte counts.

Blood (on Non-EDTA dry tubes) was centrifuged for 10 minutes at 3000 rpm and the collected sera were used for determination of:

- Glucose by GOD/POD using standard commercial kit supplied by Biodiagnostic Co.
- Nitric oxide (as nitrate) by the addition of Greiss reagent which converts nitrite into deep purple azo compound, photometric measurement of the absorbance due to this azo chromophore accurately determines nitrate concentration.
- The cytokines (TNF- α , IL-6, IL-1 β) and total IgE levels by the sandwich ELISA method with a commercially available kit (Ray Biotech, Inc. and Glory. Sciences Co., Ltd, USA respectively).

Statistical Analysis: One way analysis of variance (ANOVA) was used for multiple comparisons. When ANOVA showed significant difference ($p < 0.001$), post hoc analysis was performed with multiple comparison test ($P < 0.05$) was considered statistically significant.

RESULTS

There was significant increase in serum glucose ($p < 0.001$) with reduction in body weight ($p < 0.001$) in both STZ-induced diabetic rats and infected diabetic rats compared to normal control rats. In addition, the level of nitric oxide revealed significant increase in both groups ($p < 0.05$) and ($p < 0.001$) respectively compared to normal control. Treatment with *Carum carvi* oil for 6 weeks demonstrated significant decrease in blood glucose ($p < 0.001$) and significant increase in body weight in diabetic and diabetic infected groups compared to the untreated diabetic and diabetic infected control groups. The level of nitric oxide revealed a significant decrease in diabetic infected group after supplementation ($p > 0.001$) comparing to diabetic control as shown in Table (1).

The levels of different cytokines (IL-6, IL-1 β and TNF) and total IgE demonstrated significant increase in diabetic and infected diabetic rat groups compared to normal control one Table (2). While supplementation with *Carum carvi* oil for 6 weeks revealed decrease in different cytokines and total IgE levels compared with diabetic and diabetic infected controls. It could be noted that after

supplementation the levels tend to return to the values of normal controls. The only significant changes ($P < 0.05$) after *Carum carvi* oil supplementation were found in TNF compared to normal control and IL-6 compared to diabetic control in both diabetic and diabetic infected rats respectively.

The total leucocytes count (TLC) showed a significant increase in diabetic and diabetic infected rats ($P < 0.001$) compared to control group as observed in Table (3). Those increases in diabetic and diabetic infected groups were lowered non-significantly by supplementation with *Carum carvi*. The differential leucocytic count showed significant increase in lymphocyte percent and non-significant decrease in segmented neutrophils in diabetic and infected diabetic rats compared to normal control group. The lymphocytic count was decreased significantly, while neutrophils count was increased upon supplementation with *Carum carvi* oil comparing to diabetic infected or diabetic group and started to revert to normal control levels.

DISCUSSION

Diabetes causes the autoxidation of glucose. These changes accelerate generation of ROS and increases oxidative stress. Oxidative stress may play an important role in the development of complications in diabetes such as immune-deficiency and bacterial infection. According to the World Health Organization (WHO) [14], up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have been reported to be safe and without side-effects [15, 16].

Based on those facts, we chose a natural antioxidant compound, *Carum Carvi* oil, to study its effects on immune functions of diabetic rats and diabetic rats infected with *S.aureus*. The study used STZ diabetic rat as it is the most widely used animal model of human DM. STZ, 69% and alloxan (31%) are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally: intravenously, intraperitoneally, or subcutaneously [17, 18].

Table 1: Effect of *Carum carvi* oil supplementation on serum glucose, body weight and nitric oxide levels

Group	Serum glucose (mg/dl)	Body weight (g)	Nitric oxide (μmol/L)
Normal control	82.83 ± 9.13	250 ± 12.85	19.90 ± 1.44
Diabetic control	274.67 ± 27.22 ^a	142.17 ± 11.32 ^a	27.57 ± 6.02 ^d
Diabetic infected control	285.50 ± 19.53 ^a	169.50 ± 8.57 ^a	30.57 ± 8.59 ^a
Diabetic + <i>Carum carvi</i>	191.67 ± 16.66 ^{ab}	195.10 ± 14.46 ^a	22.0 ± 1.09
Diabetic infected + <i>Carum carvi</i>	178.33 ± 11.11 ^{ab}	198.50 ± 22.07 ^a	20.96 ± 2.21 ^b

Data expressed as mean ± SD; n = 6

a= P < 0.001 vs normal control, b= P < 0.001 vs diabetic control

c= P < 0.025 vs normal control, d= P < 0.05 vs normal control

Table 2: Effect of *Carum carvi* oil supplementation on different serum cytokines levels and serum Total IgE

Group	IL-6 (γg/dl)	IL-1β (γg/dl)	TNF (γg/dl)	Total IgE (μg/ml)
Normal control	105.16 ± 13.08	37.23 ± 6.34	19.77 ± 0.99	18.41 ± 2.74
Diabetic control	122.70 ± 10.17 ^a	47.48 ± 12.03 ^a	33.0 ± 2.96 ^a	25.72 ± 3.28
Diabetic infected control	141.9 ± 10.52 ^a	56.33 ± 14.77 ^a	34.31 ± 3.95 ^a	28.67 ± 4.03 ^a
Diabetic + <i>Carum carvi</i>	112.58 ± 2.69	40.02 ± 1.57	23.89 ± 0.89 ^c	22.97 ± 4.45
Diabetic infected + <i>Carum carvi</i>	115.49 ± 3.91 ^b	43.58 ± 6.09	26.65 ± 4.11 ^b	23.56 ± 4.79

Data expressed as mean ± SD; n = 6

a= P < 0.001 vs normal control, b= P < 0.05 vs normal control

c= P < 0.05 vs diabetic control

Table 3: Effect of *Carum carvi* oil supplementation on TLC and differential blood count

Group	TLC	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Normal control	7.97 ± 1.01	58 ± 6.1	40.33 ± 5.89	1.5 ± 0.55	-	-
Diabetic control	12.37 ± 3.13 ^a	78.17 ± 9.5 ^a	20.67 ± 4.37	1.67 ± 1.21	0.17 ± 0.41	-
Diabetic infected control	13.22 ± 6.58 ^a	78.83 ± 5.88 ^a	19.67 ± 8.89	1.67 ± 1.03	0.17 ± 0.41	0.17 ± 0.41
Diabetic + <i>Carum carvi</i>	9.6 ± 2.68	63.29 ± 10.55 ^a	34.17 ± 8.89	0.83 ± 0.75	0.33 ± 0.52	-
Diabetic infected + <i>Carum carvi</i>	11.82 ± 2.59 ^a	66.64 ± 8.91 ^a	31.0 ± 6.75	1.17 ± 0.75	0.33 ± 0.52	-

Data expressed as mean ± SD; n = 6

a= P < 0.001 vs normal control

Carum carvi is known for its anti-inflammatory and antimicrobial properties. This is attributed to the effect of its essential oils mainly Carvone and limonene [2, 3]. More recently the pharmacological properties of caraway have been reviewed with emphasis on its antioxidant properties [19, 20 new]. A dose of 50 mg/kg body weight was used in the present study. No deaths were reported in all groups. This was contradictory to that observed by Ene *et al.* [21] as they detected deaths at doses 20 and 40 mg/kg among their studied groups. They suggested that *Carum Carvi* may be toxic at high doses. We could explain the absence of deaths in our study by the shorter duration of our study (6 weeks). Diabetic rats showed symptoms of polyuria, polyphagia and polydipsia and reduced body weights which were reversed on supplementation with *Carum Carvi* oil [21]. This was attributed to reduction of blood glucose which was observed upon treatment with *Carum carvi* oil. This comes in accordance with findings of Haidari *et al.* [22]. They found that oral administration of caraway caused a significant decrease in blood glucose level of treated rats and alleviated their body weight loss.

In diabetes there is evidence of increased ROS (eg. nitric oxide) formation as a result of oxidative stress [22]. Also, during phagocytosis, macrophage response to invading microorganism generates excess nitric oxide (NO) as a result of host response against infections and inflammatory conditions [22]. In this study NO level increased in both diabetic and diabetic infected groups and was lowered by treatment with *Carum carvi*. This is attributed to the antioxidant activity of *Carum carvi* [19, 20].

It is known that NO regulates the inflammatory responses, including cytokine production, depending on its concentration [24,25]. Cytokines are a class of signaling proteins that are used extensively in immune function. IL-1β, IL6 and TNF-α are the most important immune response modifying cytokines. Diabetes is a frequent underlying medical condition among individuals with *S. aureus* infections and diabetic patients often suffer from chronic inflammation and prolonged infections [26]. This complication correlated to deregulation of immune function during diabetes in the form of increased expression of inflammatory cytokines and enhanced

generation of ROS [27]. Our results showed increase in the levels of total IgE and the most important immune response cytokines (IL-1 β , IL6 and TNF- α) in diabetic and diabetic infected rats similar to that observed in diabetic patients [28,29,30 IDOsI]. Treatment of these rats with *Carum carvi* oil reduced total IgE and cytokines levels, suggesting an overall improvement of immune function by reducing levels of proinflammatory cytokines in diabetes and diabetic infection. This is in agreement with other studies that reported potential effect of *Carum carvi* oil as an antioxidant and anti-inflammatory [4 -16].

One of the key steps during inflammation is leukocyte infiltration [24]. A number of studies have shown that diabetic patients have leukocytosis [31-35]. Our results revealed a significant increase in TLC with significant increase in lymphocytic count in both diabetic and diabetic infected rats compared to control groups. Otton *et al.* [36] suggested that a high proportion of apoptotic lymphocytes in diabetic states may explain the impaired immune function in poorly controlled diabetic patients. Our study showed decreasing TLC and lymphocytic count by supplementation with *Carum carvi*. Neutrophils or polymorphonuclear leukocytes are short-lived but abundant leukocytes. They play a central role in innate immunity. They are rapidly recruited to the site of a bacterial infection and are generally considered to be part of the “first line of defense” of the host innate immune system. Because of their sheer numbers, as well as their toxic contents and elaboration of proinflammatory cytokines, neutrophil clearance is a key to the resolution of the inflammatory response [37]. Previous studies have reported impaired bactericidal function and decreased phagocytic activity by neutrophils in diabetic patients [38]. Recently, Hanses *et al.* [39] suggested that defects in neutrophil apoptosis may contribute to the chronic inflammation and the inability to clear staphylococcal infections observed in diabetic patients. Our results revealed reduction in neutrophils percent in diabetic and infected diabetic rats indicating decreased phagocytic activity by neutrophils i.e. defect in immune response. This might explain the increased susceptibility and severity of infections in diabetic patients. Our study revealed that the antioxidant *Carum Carvi* oil supplementation decreased NO level suppressing the oxidative stress and increased the neutrophils percent improving the bactericidal process.

Certain occupations may expose workers to increased risk of diabetes as those working in glass and rubber industry. Pesticide users and workers in copper smelters

are also more prone. Those workers may benefit from the antioxidant and antibacterial of *Carum Carvi* oil. It may protect them from hazards of exposure and minimize the incidence of complications in diabetic subjects. Hence, we recommend that the potential protective effect of caraway products should be assessed in occupationally exposed subjects both diabetics and non-diabetics in future studies.

CONCLUSION

The present study concluded that *Carum Carvi* oil exhibits a potent hypoglycemic effect in STZ induced diabetic rats. It also possess an antioxidant activity that reduced the oxidative stress in STZ diabetic rats and diabetic staphylococcal infected rats. It also improved immune functions by increasing total IgE, decreasing inflammatory cytokines (IL-6, IL-1 β and TNF) and decreasing total blood count with increasing neutrophil percent. As the number of people suffering from DM has been increasing dramatically over the past few decades, special attention should be directed towards its management. Medicinal plants provide better alternatives for conventional treatments as they are generally less-toxic and affordable; yet, their safety and efficacy needs more evaluation by controlled clinical studies. Thus *Carum Carvi* oil may serve as a natural hypoglycemic antioxidant compound. It may help in attenuating diabetic complications by reducing oxidative stress and improving immune functions.

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Conflict of Interest: There is no conflict of interest.

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