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ORIGINAL ARTICLE

Immunomodulatory effects of black seeds and garlic on alloxan-induced *Diabetes* in albino rat

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Abstract
Background: Alteration in the proliferation capacity of leukocytes and in the level of some cytokines, such as TNF-α, IL-4 and IL-8 have been suggested to associate with *Diabetes mellitus* in alloxan-induced diabetic rats given the potential immunomodulatory effects of black seeds and garlic.
Aim of the work: The aim of this study was to test the effects of these agents on the immune cells in alloxan-induced diabetic rats.
Methods: To this end, *Diabetes* was induced in albino rats by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg of body weight). Diabetic rats were then fed normal diet or diet with black seeds or garlic for 28 days.
Results: The results showed significant increase in the numbers of monocytes and granulocytes, but with significant decreases in lymphocyte proliferation and the TNF-α, interleukin (IL)-4 and IL-8 levels in the diabetic group. Treatment of diabetic rats with black seeds or garlic induced significant amelioration in the numbers of monocytes and granulocytes, with significant increase in lymphocytes numbers and the TNF-α, IL-4 and IL-8 levels.
Conclusions: These results indicate the potential beneficial effects of black seeds and garlic as adjuvant treatment during treatment of *Diabetes*.

Introduction

Medicinal plants have been used to cure human illness since ancient times. Certain types of these plants are believed to promote positive health and maintain organism resistance against infection by re-establishing body equilibrium and conditioning the body tissues. Among these plants, *Nigella sativa*, an annual herbaceous plant that belongs to family *Ranunculaceae*, has been used for thousands of years in traditional medicine. Its seeds are claimed to have anticancer, antileukaemic and antimicrobial effects.[1] The seeds have been found by some authors to possess an immunopotential activity.[2] Garlic (*Allium sativum*) also has traditional dietary and medicinal applications as an antimicrobial agent.[3] Garlic is a common food spice widely distributed and used in all

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parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases.[4] Garlic is thought to have various pharmacological properties and medical applications. It is mainly consumed as a condiment in various prepared foods.[5]

Diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by limited insulin secretion or lower tissue-sensitivity to insulin. This metabolic disorder is frequently diagnosed by hyperglycaemia, lipid abnormalities and vascular complications.[6] In animals with experimental *Diabetes*, there is suppression of cellular immunity and reduced mitogenic response to several antigens.[7] It has long been recognised that the inflammatory response in diabetic patients is impaired.[8–13] Alloxan has been used to induce *Diabetes* into experimental animals. It acts through selective uptake by low affinity GLUT2 glucose transporter into the beta-cell leading to the destruction of the transporter protein by oxygen free radicals.[14] Significant reduction in the body weight gain and hyperglycaemia are present in rats after alloxan injection.[15]

Alterations in lymphocytes are a common finding in both type I and type II *Diabetes*.[16] Abnormalities of leukocyte function have been shown to occur during inflammation in alloxan-induced diabetic rats,[8] including reduced number of leukocytes in inflammatory lesions[17] and reduced production and transcription of pro-inflammatory (TNF-α) cytokines.[8,9] Lymphocyte dysfunction might be the main cause of higher incidence of infections in diabetics, since an increased number of apoptotic lymphocytes were found in alloxan-induced diabetic animals and diabetic patients.[18] Peripheral blood mononuclear cells from diabetic patients are reduced in their ability to produce cytokines[19] and the proliferate responses of CD4+ T-cells to primary protein antigens are significantly reduced.[20] Some authors suggest that metabolic disturbances and therapeutic efforts to restore glucose metabolism may underlie part of the observed changes in lymphocyte subsets in either diabetic type.[21] Elevated serum levels of IL-8, a potent chemoattractant for neutrophils and T-lymphocytes, were found in type 1 and 2 diabetic subjects, suggesting that this cytokine might contribute to the development of diabetic macroangiopathy.[22]

The aim of this work was to evaluate the beneficial effects of black seeds and garlic on the altered leukocyte number and function as well as the levels of TNF-α, IL-4 and IL-8 in alloxan-induced diabetic rats with non-diabetics.

Materials and methods

Crude extract

Black seeds and garlic cloves purchased from the local market in El-Minia, Egypt were used. The animals were allowed free access to food 50 g of seeds and cloves daily during the course of the treatment, after fasting for about 12 h.

Animals

Male albino rats (*Rattus norvegicus*), weighing 120–150 g and of average age four months, were obtained from the Biological Supply Center, Theodore Bilharz Research Institute, TBRI, Cairo, Egypt and housed under specific pathogen-free conditions and maintained on a 12-h light-dark cycle, with food and water *ad libitum*. Animals were divided into four groups (10 animals each). The first group (control) was untreated. The second, third and fourth groups were artificially *Diabetes* induced by intraperitoneal injection with alloxan (Sigma Chemical Co., USA) 120 mg/kg of body weight, freshly dissolved in 5 mmol sterile normal saline.[23] The third group was intraperitoneal injected with alloxan once during the first week and allowed free access to food 50 g of black seeds daily during the course of the treatment, after fasting for about 12 h. The fourth group was treated similarly to the third group but with garlic instead of black seeds. Rats with blood glucose levels $\geq$250 mg/dl were considered to be diabetic. Plasma glucose levels in the control animals remained normal throughout the study.

Quantification of serum cytokines

The concentrations of TNF-α, IL-4 and IL-8 in collected serum samples were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturer's instructions (R&D Systems Inc., Minneapolis, MN, USA). The sensitivity of the assays was 15 pg/ml.

Leukocyte differential count

Freshly collected blood samples of about 20 μl were spread on clean slides as a thin film using another smooth-edged glass slide. Each blood smear was left to air dry before being fixed with methanol for 2–3 min and then labelled by the number of the animal. Blood smears were stained with 10% Giemsa's stain (Aldrich) in buffered distilled water containing 0.021 M Na_2HPO_4/0.015 M KH_2PO_4. pH 7–7.2 for 30 min and kept away from sunlight. After that, the stain was removed by gentle washing with distilled water and the slides were air-dried at room temperature.[24] Using light microscopy at 400$\times$ magnification, different types of blood leukocytes were recorded. At least double smears for each blood sample were counted.

Statistical analysis

Data were analysed using SPSS program version 13.0. Statistical analysis of the obtained data was performed using one way analysis of variance (ANOVA) test followed by least square differences (LSD) analysis for comparison between means. Results were expressed as mean $\pm$ standard error (SE). Values of $P < 0.05$ were considered statistically significant, while values of $P > 0.05$ were considered statistically non-significant.

Results

Quantification of TNF-α in sera

The TNF-α ELISA values demonstrated that the control group (1780.39 $\pm$ 52.621 pg/ml) is higher than the diabetic group