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Immunomodulatory Effect of *Nigella Sativa* Oil in the Disease Process of Type 1 Diabetic Rats

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ABSTRACT

This study was carried out to investigate the potential immunomodulatory effect of administration of *Nigella sativa* oil (NSO) in the autoimmune disease process of type 1 diabetes mellitus (IDDM). By using of ELISA kits, the levels of anti- islet cell antibodies (ICA), Pan T- lymphocytes (CD90), Pan B-lymphocytes markers (CD19), and innate cell marker (CD11b) were performed in addition to assessment of blood glucose and serum insulin through the experiment. 24 rats were included in this study. They were equally divided into four groups as following; (1) control group; (2) diabetic non treated group, (3) and (4) were two treated groups with different dose of oil (0.2-0.4 ml/kg) respectively, for a period of 30 consecutive days. IDDM increased the levels of serum glucose, levels of T, B lymphocytes markers, innate cells marker, and ICA and decreased serum insulin level, meanwhile treatment of diabetic rats with NSO (especially at high doses) significantly decreased the levels of all immunological parameters. Beside it significantly resulted in elevation of serum insulin level. The data may provide new strategies for using NSO to be recommended in the clinical management of IDDM.

Keywords*: Nigella sativa*, Type1 diabetes mellitus, CD markers, Anti islet cell antibody, serum insulin.

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INTRODUCTION

Type 1 diabetes mellitus (IDDM) is an organ-specific autoimmune disease. It is a chronic disease resulting when the immune system attacks and destroys the insulin-producing ß cells in the pancreatic islets of Langerhans of the pancreas [1]. This destruction of ß cells and occurrence of disease is resulting of uneasiness immune regulation [2].

IDDM is characterized by the presence of antibody (humoral) and T-cell (cellular) responses to islet proteins. At the presentation of clinical type 1 diabetes 80-90% of beta-cells are destroyed [3]. In almost all prediabetic individuals and patients with newly diagnosed type 1 diabetes before or at the diagnosis of clinical disease, autoantibodies to beta-cell antigens are seen [4,5]. 70- 90% of patients with newly diagnosed type 1 diabetes have been observed with islet cell autoantibodies (ICA) [6,7], which makes them one of the most sensitive humoral markers of clinical type 1 diabetes [8].

Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure [9]. A single injection of streptozotocin (STZ) is widely used to generate a rat model of type I diabetes, which results from the selective toxicity of STZ towards the insulin-producing β-cells in pancreatic islets [10]. A single dose of 65mg/kg of STZ are able to induced IDDM to experimental animals [11].

Modulation of the immune system denotes to any change in the immune response that can involve induction, expression, amplification or inhibition of any part or phase of the immune response. Thus, immunomodulator is a substance used for its effect on the immune system. The potential uses of immunodulators in clinical medicine include the reconstitution of immune deficiency and the suppression of normal or excessive immune function [12]. The uses of natural products as *Nigella sativa* have culminated in the development of a variety of drugs that are medically proven for their therapeutic effectiveness against a wide range of diseases [13].

Nigella sativa is an annual flowering plant belongs to the family Ranunculaceae. The seed of Nigella sativa is known by many different names like black seeds or black cumin. It has been traditionally used for variety of applications including treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support [14].

In several studies the effect of *Nigella sativa* oil (NSO) on the immune system has been investigated [15, 16]. All these studies have shown that the oil of N. sativa inhibits many inflammatory mediators, and, may be useful in ameliorating inflammatory and autoimmune conditions. However, there is no report about the possible protective effect of the NSO against the autoimmunity process of type 1 diabetes itself. Classically, investigations were mostly confined to glucose–insulin circle. However, in this present study, the potential

immunomodulatory effects of NSO on the disease process of type 1 diabetes in STZ diabetic rats were evaluated.

MATERIALS AND METHODS

Experimental Animals

Male Sprague-Dawley rats with an average weight of 150-250g and an average age of 12-16 weeks were used throughout the experiments. The animals were obtained from Nano Life Quest Company. Ethical clearance for performing the experiment on animals was approved by Animal Care and Use Committee (ACUC), Faculty of Medicine, Universiti Teknologi MARA UiTM (ACUC-2/11). The rats were acclimatized for a period of 21 days in the Laboratory Animals Care Unit (LACU) Faculty of Medicine, Sg Buloh Campus, Universiti Teknologi MARA UiTM. A standard environmental condition such as temperature (20-22C0), relative humidity (45-55%) and 12 hrs. dark/light cycles was maintained. The animals were fed daily with rodent pellet diet and tap water ad-libitum under strict hygienic conditions.

Chemicals

The Streptozotocin (STZ) (2-deoxy-2-({[methyl (nitroso) amino] carbonyl} amino)-β-D-glucopyranose) used in the present study was purchased from Sigma, Germany.The Nigella sativa oil was produced by Kausar, Iran. It is a pure preparation, with no additive. The oil was administered once a day by intraperitoneal (i.p) injection at doses of either 0.2 ml/kg or 0.4 ml/kg for 30 days.

Induction of type 1 diabetes

Type 1 diabetic was induced to overnight fasted animals by intraperitoneal injection with a single dose of STZ (65 mg/kg body weight) to all animal groups except the normal control group (25). STZ was dissolved in sodium citrate buffer solution (PH 4.5) immediately before use. The rats with blood glucose above13.9 mmol/L (250 mg/dL), which lasted for at least three days, were considered as type 1 diabetic rat [17-18].

Experimental design

The experimental animal groups were divided into 4 groups (6 rats each). The groups are: Group A (Normal control group received 0.1 sodium citrate buffer), Group B (Diabetic control group treated with STZ only 65 mg/kg), Group C (Diabetic rats received i.p. NSO low dose 0.2 ml/kg), and Group D (Diabetic rats received i.p. NSO high dose 0.4 ml/kg).

Fasting blood glucose measurement

Blood glucose was tested every morning (at 8 am) through 4 weeks of the experimental period. Blood was collected from the tail of fasting (14 h) animals. The blood glucose level was

tested by using of glucometer purchased from (Roche, USA).

Samples collection and serological study

After completion of 30 days of experimental protocols, blood samples were collected from overnight fasted rats. The animals were anesthetized in a chamber containing diethyl ether. Blood was collected from the heart by the cardiac puncture. Immediately after collection, the blood was transferred into fresh tube and centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored at –80 C° until further analysis. Serum was assayed for levels of anti- islet cell antibodies (ICA), Pan T- lymphocytes (CD90), Pan B-lymphocytes markers (CD19), and innate cell marker (CD11b), in addition to measure the serum insulin level. All tests were performed by using enzyme-linked immunosorbent assay (ELISA) (USCNK, CHINA).

Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for the positive and negative control groups. Results are expressed as mean ± SD. The significant differences among values were analyzed using by one way analysis of variance (ANOVA) carried out by SPSS 16 software followed coupled with post-hoc least p-value of < 0.05 was considered as statistically significance

RESULTS AND DISCUSSION

Biochemical and Immunological Results

The diabetic group (Group B) exhibited hyperglycemia, with significantly increased in fasting blood glucose (FBS) level ($P < 0.05$) as compared with control (Group A) at different time points (figure.1). However, after 10 days treatment with NSO (Group C and D), there was a slight, but non-significant ($P > 0.05$), decrease in serum glucose levels compared with those in untreated diabetic group (Group B). By the end of the experimental period, blood glucose levels in Group C and D showed significant decrease ($P < 0.05$) (figure.1).

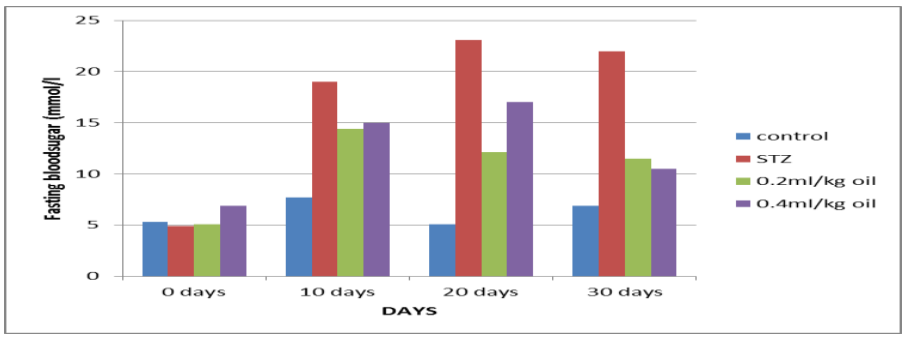

Fig 1. Levels of FBG for, GA; control group, GB; untreated diabetic rats; GC, diabetic rats treated with (0.2 ml/kg) NSO .GD, diabetic rats treated with (0.4 ml/kg) NSO. Data are expressed as mean ±SD.

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The diabetic group (Group B) exhibited significantly decreased serum insulin levels ($p < 0.05$) compared to control (Group A). However, treatment of diabetic rats with the NSO (Group C and Group D) resulted in a significant increase in serum insulin levels compared to the untreated diabetic group (Group B) after 30 days of treatment ($p < 0.05$) (Figure 2).

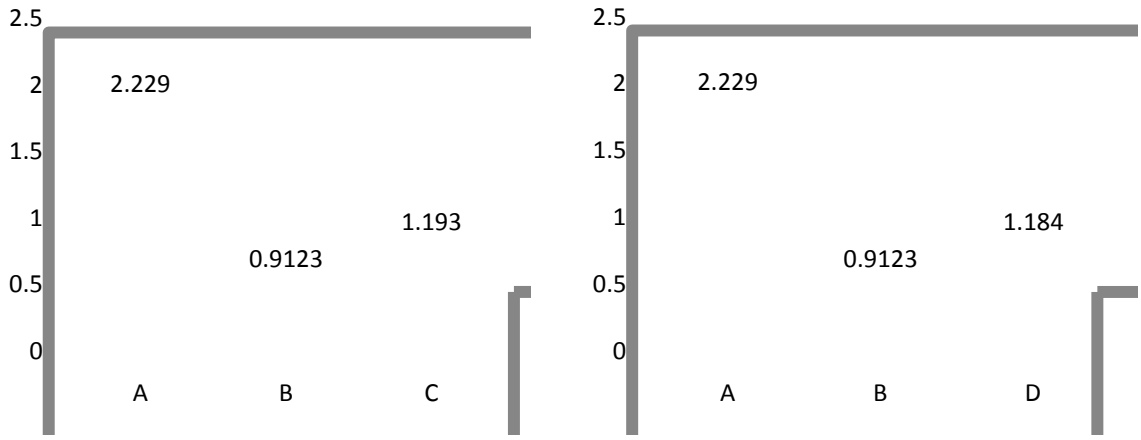

Fig 2. Effects of NSO administration on Insulin level. Group A, control group; Group B, untreated diabetic rats; Group C,D; diabetic rats treated with 0.2 and 0.4 ml/kg NSO respectively. Data are expressed as mean ±SD.

In STZ diabetes group there was increase the levels of anti-islet cell auto antibodies ICA, however, in group C, STZ diabetic rats treatment with (0.2 ml/kg i.p) NSO ICA level shows a slight decrease compared to group B (STZ diabetic untreated).However, a significant decreased in level of ICA was observed in group D diabetic rats treated with 0.4 ml/kg NSO ($P < 0.05$) after 30 days treatment (Figure 3). The same result were observed with other immunological markers (CD19, CD90, and CD11) which increased after diabetic induction but by the end of the experiment, high dose of NSO treatment significantly caused decrease in all elevated markers. Figures 4, 5, and 6 respectively

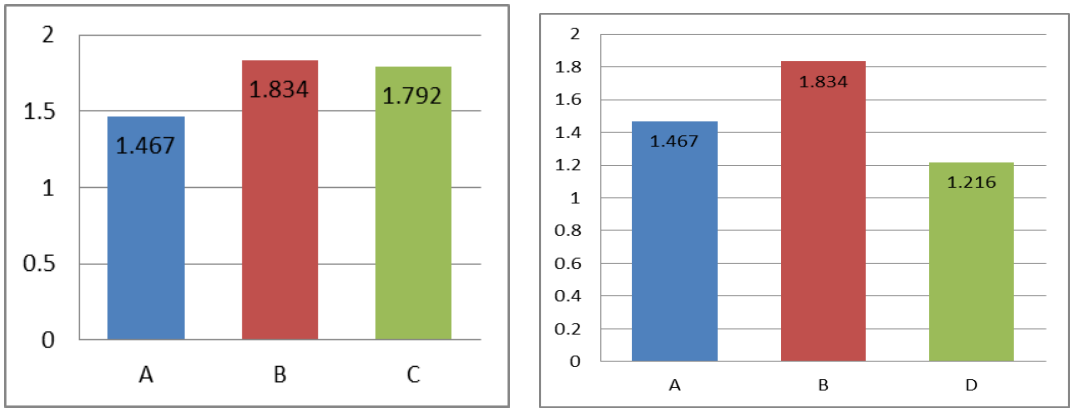

Fig 3. Effect of NSO administration on ICA level production. Group A, control group; Group B, untreated diabetic rats; Group C,D, diabetic rats treated with 0.2 and 0.4 ml/kg NSO respectively. Data are expressed as mean ±SD.

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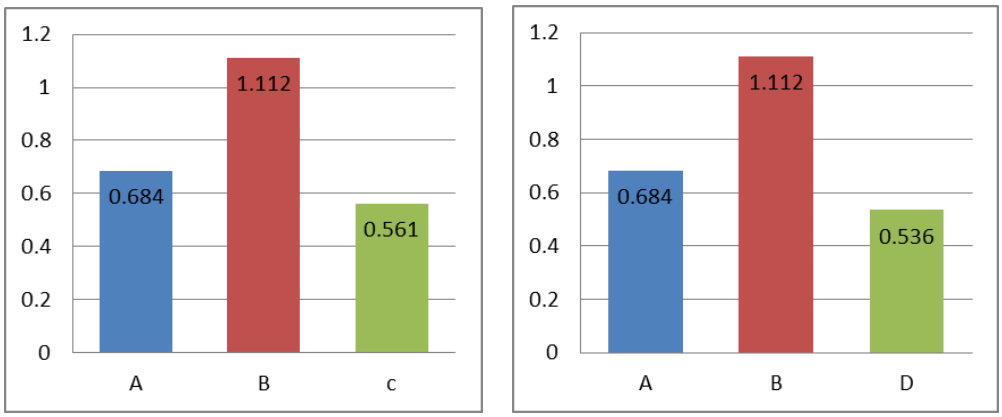

Fig 4. Effect of NSO administration on the CD19 levels. Group A, control group; Group B, untreated diabetic rats; Group C,D, diabetic rats treated with 0.2 and 0.4 ml/kg NSO respectively. Data are expressed as mean ±SD.

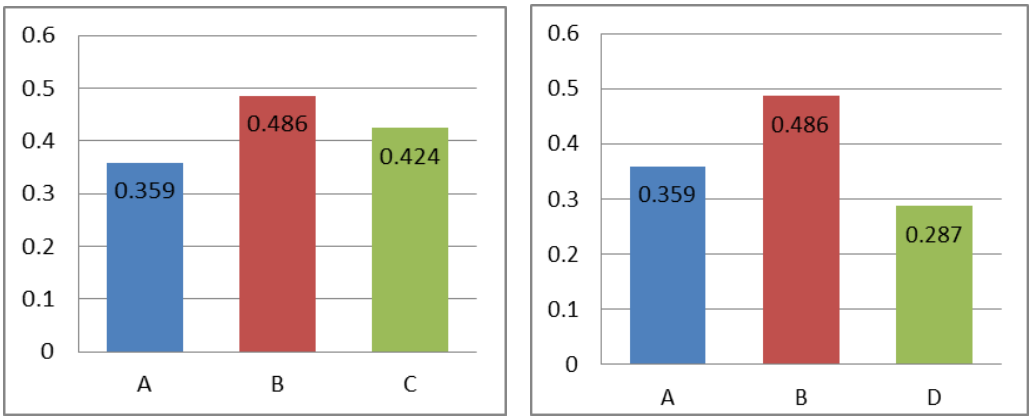

Fig 5. Effect of NSO adminstration on the CD90 levels. Group A, control group; Group B, untreated diabetic rats; Group C,D, diabetic rats treated with 0.2 and 0.4 ml/kg NSO respectively. Data are expressed as mean ±SD.

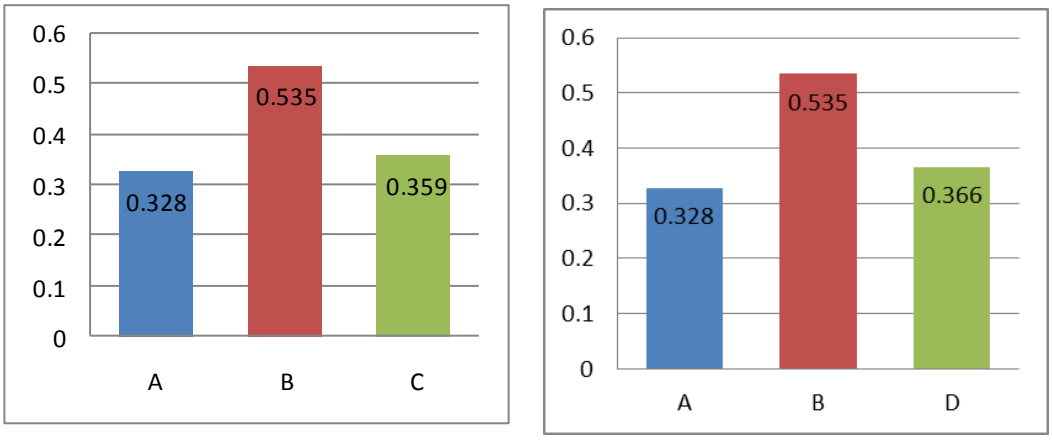

Fig 6: Effect of NSO administration on the CD11b levels. Group A, control group; Group B, untreated diabetic rats; Group C,D, STZ-diabetic rats treated with 0.2 and 0.4 ml/kg NSO respectively. Data are expressed as mean ±SD.

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DISCUSSION

This study, according to the best of our knowledge is the first to provide experimental evidence demonstrating that *N. sativa* has immunomodulatory properties against the autoimmunity process of type 1 diabetes.

N. sativa has been used in traditional medicine as antidiabetic and many studies have been showed that *N. sativa* can decrease blood glucose level in STZ induced diabetes [19, 20]. Meanwhile, evidences from this current study have showed that treatment with *N. sativa* oil (especially at the high dose) reduces levels of anti-islet cell antibodies ICA which are the main antibodies produced in autoimmune process of the disease and that caused reduced the level of all the immunological markers (pan B cell marker (CD19), pan T cell marker (CD90) and (CD11b) pan innate cell marker) which have been evaluated.

IDDM is a debilitating chronic disease that impairs production and secretion of the key hormone insulin and alters blood sugar metabolism. Insulin is synthesized and secreted by pancreatic islet cells or Islets of Langerhans [21]. The disruption of insulin synthesis is caused by immunological destruction of the islet cells by autoantibodies in IDDM patients [22]. During this period, the affected individuals exhibit the diminishing early-phase release of insulin in response to an intravenous/oral glucose challenge. In the majority of cases, these individuals carry circulating islet cell autoantibodies (ICA) [21].

Many Studies have shown that N. sativa and TQ have effect on immune system; they are able to inhibit many inflammatory mediators and could ameliorate inflammatory and autoimmune conditions [23, 24]. Based on the results obtained from the in vitro experiments *N. sativa* constituents have shown to down regulate B cell-mediated immunity [25]. N. sativa seed was studied on the antigen-specific response induced by vaccinating rats with the typhoid TH antigen. In that study, treatment with N. sativa oil induced about 2-fold decrease in the antibody production in response to typhoid vaccination as compared to the control rats [26]. Mohamed, (2005) had reviewed that certain constitutions of N. sativa oil possess potent potentiating effects on the cellular immunity, while other constituents possess suppressor effects on B cell-mediated immunity. These findings suggest also that the stimulatory effects of N. sativa on the cellular immunity are dependent on the nature of the immune response. However, in this current study we clearly found that N. sativa oil caused suppressions in both cellular and humoral immunity [27].

In summary, NSO lead to decrease in the serum levels of autoantibodies (ICA) against the β cells which are the main antibody produced among autoimmune process of IDDM in addition lead to decreased in the level of T, B lymphocytes and innate cell markers and lastly lead to stop the disease process of autoimmunity occurs in this disease.

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CONCLUSION

These experimental results indicate the immunomodulatory effects of this plant against autoimmune reactions occurred in IDDM and immune defense in IDDM can be significantly improved by the administration of NSO. The data may provide new strategies for using NSO to be recommended in the clinical management of IDDM.

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Disclosure: There are no conflicts of interest to declare.

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