

Mechanisms of the hypoglycaemic and immunopotentiating effects of *Nigella sativa* L. oil in streptozotocin-induced diabetic hamsters

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Abstract

The aim of this study was to elucidate the mechanisms underlying the hypoglycaemic effect of *N. sativa* oil (*Nigella sativa* oil) in streptozotocin (STZ)-induced diabetic hamsters, in terms of hepatic glucose production, and to investigate the possible immunopotentiating effect of *N. sativa* oil on peritoneal macrophages. Diabetes was induced by intraperitoneal injection of 65 mg/kg body weight of STZ. Treatment with *N. sativa* oil commenced 6 weeks after induction of diabetes at a dose of 400 mg/kg body weight by gastric gavage. Isolated hepatocytes were collected using collagenase to determine liver glucose production. Phagocytic activity was evaluated by injection of fluorescent latex (2 µm diameter) intraperitoneally, followed 24 h later by collection of peritoneal macrophages. *N. sativa* oil reduced blood glucose from 391 ± 3.0 mg/dl before treatment to 325 ± 4.7, 246 ± 5.9, 208 ± 2.5 and 179 ± 3.1 mg/dl after the first, second, third and fourth weeks of treatment, respectively. Hepatic glucose production from gluconeogenic precursors (alanine, glycerol and lactate) was significantly lower in treated hamsters. Treatment with *N. sativa* oil significantly increased the phagocytic activity and phagocytic index of peritoneal macrophages and lymphocyte count in peripheral blood compared with untreated diabetic hamsters. Our data indicate that the hypoglycaemic effect of *N. sativa* oil is due to, at least in part, a decrease in hepatic gluconeogenesis, and that the immunopotentiating effect of *N. sativa* oil is mediated through stimulation of macrophage phagocytic activity either directly or via activation of lymphocytes.

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1. Introduction

Increased utilization of medicinal plants became a World Health Organization (WHO) policy in 1970. Plants and herbs are chemical factories that directly provide about 25% of currently used drugs and another 25% of drugs comprise chemically altered natural products (De Smet, 1997). *Nigella sativa* L., commonly known as black seed, belongs to the botanical family *Ranunculaceae*. It has been in use in many Middle Eastern countries as a natural remedy for over 2000 years (Swamy and Tan, 2000). Black seed components display a remarkable array of biochemical, immunological and pharmacological actions, including bronchodilatory (Boulos, 1983), anti-in-

flammatory (Houghton et al., 1995), antibacterial (Agarwal et al., 1979), hypoglycaemic (Al-Hader et al., 1993) and immunopotentiating (Haq et al., 1999) effects.

The pathogenesis of diabetes mellitus and the possible management of diabetes in animals by oral hypoglycaemic agents have been extensively investigated in recent years (Ribes et al., 1986). The WHO expert committee on diabetes mellitus recommendations of 1980 (WHO, 1980) included investigation of hypoglycaemic agents from plants used in traditional medicine. *N. sativa* oil have been used for treatment of experimentally induced diabetes in animals based on its combined hypoglycaemic and immunopotentiating effects that help in ameliorating the impaired immunity and infections associated with diabetes (Al-Hader et al., 1993; Deresinski, 1995). However, the mechanism underlying the hypoglycaemic effect of *N. sativa* oil is unclear and its immunopotentiating effect has never been investigated in diabetic animals. The

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present study was designed to investigate the mechanism(s) of the hypoglycaemic effect of *N. sativa* oil, especially with respect to hepatic gluconeogenesis, and to investigate its possible immunopotentiating effects in diabetic hamsters.

2. Materials and methods

2.1. Plant material

Nigella sativa L. oil extract was obtained from Kahira Pharmaceuticals. Oil was dissolved by the initial addition of dimethyl sulphoxide (DMSO), followed by the addition of normal saline. Oil was administered at a dose of 400 mg/kg body weight once daily by gastric gavage for 4 weeks.

2.2. Animals

Eight-week-old male Syrian hamsters (80–120 g body weight) were obtained from a commercial laboratory animal company (Gifu, Japan), placed in stainless steel cages and maintained under suitable lighting, temperature and hygienic conditions. Well-balanced food rations and drinking water were provided. The hamsters were observed for 12 days prior to experimentation. Animals were anaesthetized with diethyl ether and then sacrificed by exsanguination from the carotid arteries. All surgical procedures and pre- and post-operative care of the animals were approved by the Gifu University Animal Care and Use Committee, in accordance with the Japanese Department of Agriculture guidelines and all efforts were made to minimize animal suffering and the number of animals used.

2.3. Streptozotocin-induced diabetes

Streptozotocin (STZ) was obtained from Sigma Chemical (St. Louis, MO). Hamsters were injected intraperitoneally with a single dose of STZ (65 mg/kg body weight) in a volume of 0.5 ml/hamster. STZ was dissolved in sodium citrate buffer solution (pH 4.5; Wako Pure Chemicals, Osaka, Japan) immediately before use. Animals were fasted for 6 h prior to injection of STZ (Karnieli et al., 1981). Control animals were injected with the buffer solution alone. All animals were then maintained for 6 weeks on ad libitum food and water with monitoring of blood glucose, body weight and food and water consumption before commencement of treatment with *N. sativa* oil.

2.4. Blood tests

Blood samples were collected from the medial canthus of the eye and heart puncture into sterilized tubes

for serum separation and into tubes containing heparin for hematological studies using standard methods (Riley, 1960). Blood glucose was measured according to method adopted previously by Miwa et al. (1972) using a glucose kit (enzymatic method) (Wako). Total glycated haemoglobin was estimated according to a standard technique (Bunn et al., 1976) using a total glycated haemoglobin kit (Sigma).

2.4.1. Estimation of liver glucose production

Hepatic glucose production was estimated using the methods described previously (Pogson and Smith, 1975; Al-Awadi et al., 1991). First, we prepared hepatocytes from STZ-diabetic hamsters. For this purpose, hamsters were fasted for 24 h, and then anaesthetized by intraperitoneal injection of 50 mg/kg body weight of phenobarbitone (Sigma, containing 6000 units/kg heparin dissolved in normal saline). Cannulation for perfusion was performed by insertion of a cannula through the portal vein as the inlet and inferior vena cava as the outlet. Perfusion was performed using 50 ml calcium-free Krebs buffer (+1 mg/ml EDTA) at flow rate of 25 ml/min. EDTA was then removed by washing the liver with 100 ml calcium-free Krebs buffer. Collagenase (Sigma) was then added to the medium (0.1% w/v). Medium leaking from the surface of the liver indicated completion of perfusion. Liver dispersion was performed at 36 °C and the isolated cells were washed by Krebs buffer containing 2% bovine serum albumin (w/v) (Sigma). Finally, cells were suspended in 100 ml of the same buffer. Throughout the procedures, the buffered medium was bubbled with 5% CO₂–95% O₂ gas mixture. Viability of the cells was tested by the trypan blue exclusion test. Trypan blue solution was prepared in saline to a final concentration of 0.04%. To determine the gluconeogenic activity of the isolated hepatocytes, the cells were incubated at a density of 2×10^6 cells/ml at 37 °C under 5% CO₂ in Krebs buffer containing calcium (pH 7.4) in a total volume of 2 ml. Glycerol, lactate and alanine (Sigma) were used separately as substrates at final concentrations of 10 mM. The reaction was terminated after 2 h by the addition of 0.2 ml 20% perchloric acid (Sigma). Neutralized samples were assayed using a glucose oxidase kit (Sigma).

2.5. Determination of phagocytic activity of hamster peritoneal macrophages

2.5.1. Flow cytometry

Fluorescent latex particles (2.5% latex solids, 2.0 µm in diameter; Polyscience, Warrington, PA) were injected intraperitoneally (0.5 ml fluorescent particles [2×10^8]) into each hamster. After 24 h, hamsters were sacrificed under light ether anaesthesia. Approximately 7 ml of cold serum-free RPMI 1640 medium (Whittaker, Walkersville, MD) were injected twice intraperitoneally

through the exposed peritoneum. After gentle massage, peritoneal fluid containing cells was harvested and centrifuged at 250g for 10 min at 4 °C. The cell pellet was then reconstituted with 3 ml of PBS (pH 7.4) containing 10% bovine calf serum (Multi Ser, Australia). Examination of the collected macrophages was performed using a flow cytometer (Immunocytometry System, Becton–Dickinson, Mountain View, CA) according to the method described previously (Carleton et al., 1986).

2.5.2. Fluorescence microscopy

A fluorescence microscopy study was performed by counting 200 macrophages and estimating the number of phagocytosed fluorescent particles in each cell.

2.6. Differential leukocyte counts

Thin blood films were prepared from freshly collected blood, and then dried and fixed with absolute methyl alcohol. Staining was performed using Giemsa stain (Sigma). Differential counts were performed under light microscopy using an oil immersion lens. The percentages of lymphocytes, neutrophils, band cells, monocytes, basophils and eosinophils were evaluated according to standard techniques (Coles, 1986).

2.7. Statistical analysis

Data were expressed as means \pm SEM. Differences between groups were examined for statistical significance using the ANOVA and Student's *t* test. A *p* value less than 0.05 denoted the presence of a statistically significant difference (Zar, 1974).

3. Results

3.1. Effect of *N. sativa* oil on blood glucose level and total glycated haemoglobin

After induction of STZ-diabetes in hamsters by 6 weeks, treatment with *N. sativa* oil and DMSO was started and continued for 4 weeks. Significant falls in plasma glucose levels ($p < 0.001$) was found in diabetic hamsters treated with 400 mg/kg *N. sativa* oil after 1, 2,

3 and 4 weeks of administration compared to diabetic-untreated hamsters. Diabetic hamsters treated with DMSO showed non-significant decrease in plasma glucose level compared to diabetic-untreated ones (Table 1). Total glycated haemoglobin was $5.9 \pm 0.9\%$ in normal non-diabetic hamsters. Significant decrease ($p < 0.01$) in the level of total glycated haemoglobin was found in diabetic hamsters treated with *N. sativa* oil for 4 weeks (the level reached $12.1 \pm 1.0\%$) compared to diabetic-untreated hamsters (where the level was $17.3 \pm 0.7\%$). Non-significant decrease was found in the level of total glycated haemoglobin in diabetic hamsters treated with DMSO ($17.0 \pm 0.5\%$) compared to diabetic-untreated ones.

3.2. Effect of *N. sativa* oil on hepatic gluconeogenesis and glucose production

Hepatocytes isolated from *N. sativa* oil-treated hamsters showed a significant fall in glucose production in response to incubation for 2 h with gluconeogenic precursors [alanine, glycerol ($p < 0.001$) and lactate ($p < 0.05$)], compared with hepatocytes isolated from untreated diabetic animals (Fig. 1).

3.3. Immunopotentiating effects of *N. sativa* oil

3.3.1. Phagocytic activity of peritoneal macrophages

The phagocytic index (number of macrophages that phagocytosed fluorescent particles) and phagocytic rate (number of fluorescent particles in each macrophage) were significantly higher in STZ-diabetic hamsters treated with *N. sativa* oil compared with untreated diabetic animals, as demonstrated by fluorescence microscopy. This was represented by significant decrease in the number of macrophages that contain 1, 2 and 3 fluorescent particles and significant increase in the number of macrophages that contain 4–20 fluorescent particles in diabetic animals treated with *N. sativa* oil as shown in Table 2 and Fig. 2. Non-significant changes in the phagocytic rate in diabetic animals treated with DMSO. Flow cytometric analyses also showed a significant increase in macrophage phagocytic activity as indicated by

Table 1
Changes in fasting plasma glucose levels in diabetic hamsters during 4 weeks of treatment with *Nigella sativa* oil

	Normal	Diabetic-untreated	Diabetic treated with DMSO	Diabetic treated with <i>N. sativa</i>
Pre-treatment (mg/dl)	99 \pm 3.2	389 \pm 4.5	395 \pm 4.0	391 \pm 3.0
First week (mg/dl)	101 \pm 4.2	405 \pm 3.3	397 \pm 4.5	325 \pm 4.7*
Second week (mg/dl)	99 \pm 4.1	409 \pm 3.6	395 \pm 3.2	246 \pm 5.9*
Third week (mg/dl)	103 \pm 3.5	408 \pm 4.6	391 \pm 2.9	208 \pm 2.5*
Fourth week (mg/dl)	101 \pm 3.4	410 \pm 4.0	398 \pm 3.1	179 \pm 3.1*

Values are means \pm SEM of seven hamsters in each group.

* $p < 0.001$, compared with the corresponding value in untreated diabetic hamsters.

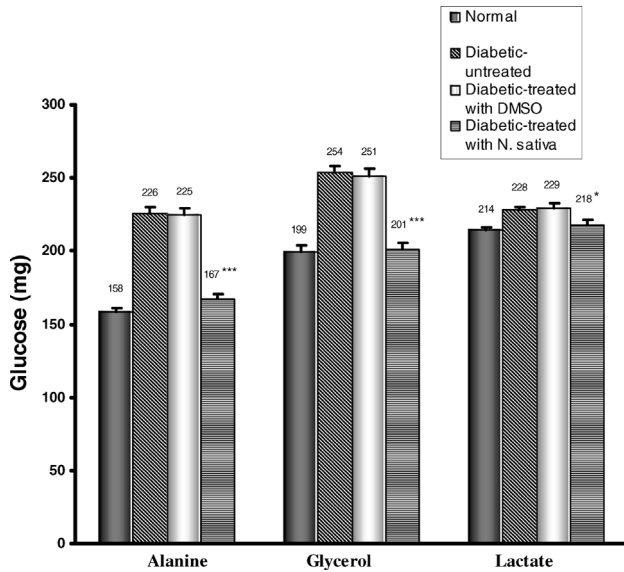


Fig. 1. Changes in hepatic glucose production from alanine, glycerol and lactate in STZ-diabetic hamsters after treatment with *Nigella sativa* oil. Values are means \pm SEM of seven hamsters. * $p < 0.05$, *** $p < 0.001$ compared with the corresponding value in untreated diabetic hamsters.

the increase in fluorescence intensity after treatment with *N. sativa* oil (Fig. 3).

3.3.2. Differential leukocyte count

A significant increase ($p < 0.01$) in the percentage of blood lymphocytes was observed in the *N. sativa* oil-treated group ($74.7 \pm 2.6\%$) compared with untreated diabetic animals ($54.0 \pm 2.6\%$).

4. Discussion

The results of this study clearly demonstrate that oral administration of oil extracted from *N. sativa* seeds produced a significant, consistent and time-dependent decrease in blood glucose levels in STZ-induced diabetic hamsters. This finding might explain the use of *N. sativa* seeds, in addition to other plants, in preparations widely used as anti-diabetic remedies in Middle East folk medicine. The hypoglycaemic effect observed in the present study became more significant after daily administration of the extract for one month to the diabetic animals.

Our results also showed that treatment with *N. sativa* oil significantly reduced total glycated haemoglobin, compared with the untreated diabetic animals. Total glycated haemoglobin is an important parameter used to monitor response to glucose-lowering therapy and long-term blood sugar control, as it reflects the average blood sugar concentration over an extended period of time and remains unaffected by short term fluctuations in blood glucose levels (Gabbay et al., 1977). The decrease in total glycated haemoglobin levels observed in our study reflects the adequate and effective action of *N. sativa* oil in long-term reduction of diabetic hyperglycaemia. Non-significant changes were observed in blood glucose level and total glycated haemoglobin. These results are in accordance with Al-Hader et al. (1993) who found non-significant changes in hyperglycaemic rabbits after treatment with DMSO.

The mechanism of action of *N. sativa* oil seems to be complex as it consists of both volatile and non-volatile

Table 2

Phagocytic index and phagocytic rate in STZ-diabetic animals after 4 weeks treatment with *Nigella sativa* oil using fluorescent microscope

No. of phagocytosed particles	Phagocytic rate in normal hamsters	Phagocytic rate in diabetic hamsters	Phagocytic rate in DMSO-treated hamsters	Phagocytic rate in <i>N. sativa</i> oil-treated hamsters
0	4 \pm 1.2	20 \pm 1.1	20 \pm 0.1	10 \pm 1.3*
1	3 \pm 1.3	48 \pm 1.5	47 \pm 1.0	12 \pm 1.2*
2	5 \pm 1.1	34 \pm 0.6	34 \pm 1.7	14 \pm 1.3*
3	10 \pm 1.4	31 \pm 1.2	32 \pm 2.2	15 \pm 1.4*
4	15 \pm 1.7	10 \pm 1.3	11 \pm 1.5	20 \pm 1.1**
5	30 \pm 0.8	15 \pm 1.2	14 \pm 0.3	25 \pm 1.3**
6	27 \pm 1.3	10 \pm 1.2	10 \pm 1.2	25 \pm 1.5**
7	30 \pm 1.5	5 \pm 1.3	6 \pm 1.0	19 \pm 0.5**
8	30 \pm 1.6	3 \pm 1.1	3 \pm 0.1	15 \pm 1.5**
9	4 \pm 1.3	4 \pm 1.2	4 \pm 1.6	11 \pm 1.1**
10	15 \pm 1.7	4 \pm 1.3	4 \pm 0.5	6 \pm 1.3*
11	5 \pm 1.5	5 \pm 1.7	3 \pm 1.9	3 \pm 1.2
12	2 \pm 1.1	2 \pm 1.1	3 \pm 0.1	6 \pm 1.8*
13	4 \pm 1.2	2 \pm 1.5	2 \pm 0.9	4 \pm 0.6
14	0	4 \pm 1.2	4 \pm 1.3	0
15	8 \pm 1.3	0	1 \times 0.1	5 \pm 1.2
16	0	0	0	6 \pm 0.8***
17	4 \pm 1.2	0	0	4 \pm 0.5***
20	4 \pm 1.3	0	0	0

Values are means \pm SEM of seven hamsters in each group.

$p < 0.001$, * $p < 0.05$, ** $p < 0.01$, compared with the corresponding value in untreated diabetic hamsters.

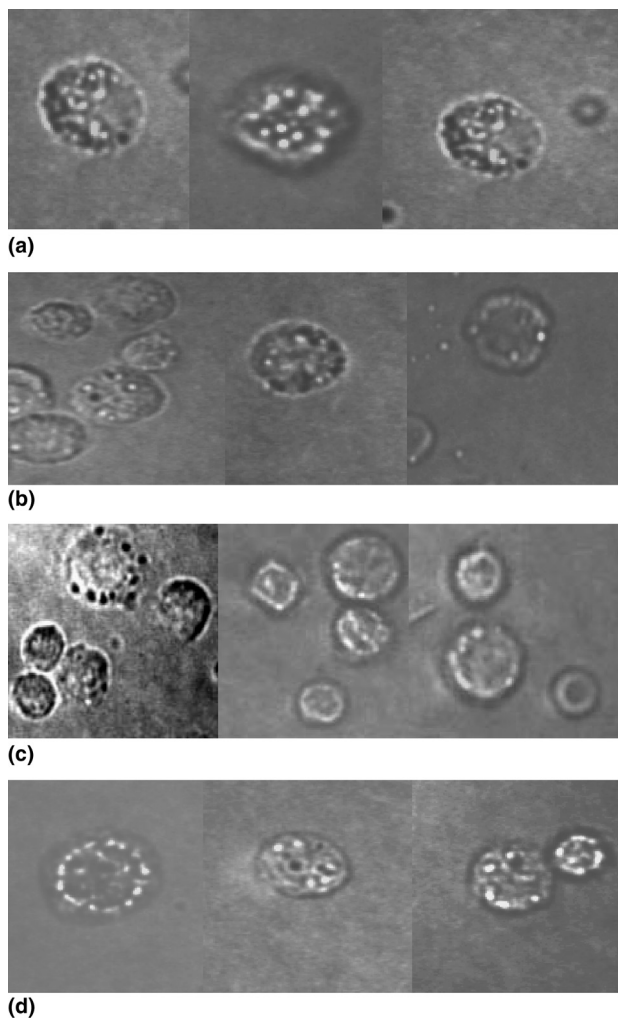


Fig. 2. Phagocytosed fluorescent particles in macrophages of normal (a), diabetic (b), DMSO-treated (c) and *Nigella sativa*-treated groups (d) (fluorescence microscopy, magnification 1500 \times).

oils, and various actions of *N. sativa* oil have been observed, including bronchodilation, antiparasitic and antihistamine effects (Hedaya, 1995; Chakravarty, 1993). Measurement of the effect of *N. sativa* oil on gluconeogenesis and liver glucose production helps to clarify part of this hypoglycaemic mechanism since hepatic glucose production through gluconeogenesis is known to contribute significantly to hyperglycaemia in diabetic patients (Ishikawa et al., 1998). Our studies on isolated hepatocytes demonstrated a significant decrease in glucose output from gluconeogenic precursors (alanine, glycerol and lactate) in *N. sativa* oil-treated compared to untreated animals. This significant decrease in liver glucose output suggests that the observed anti-diabetic action of *N. sativa* oil is at least partially mediated through a decrease in hepatic gluconeogenesis.

Increased glycolysis in peripheral tissues and/or inhibition of the release of counter-regulatory hormones (e.g., glucagon, cortisol and growth hormone) are pos-

sible contributory mechanisms that may be considered and require further investigation.

Impairment of the immune system in diabetic subjects is well described and its relevance to alteration of the phagocytic properties of monocytes/macrophages has been proposed (Class et al., 1986; Brownlee et al., 1988). Objective quantitation of phagocytic activity includes two types of parameters, the phagocytic index (number of macrophages that phagocytose fluorescent particles) and the phagocytic rate (number of phagocytosed fluorescent particles in each cell) according to Bassoe et al. (1983). In the present study, the phagocytic index and phagocytic rate were improved in diabetic animals treated with *N. sativa* oil for one month, which was represented by significant increase in the number of macrophages that contain from 4 to 20 fluorescent particles compared with decreased values in untreated diabetic hamsters. These results clearly demonstrate that *N. sativa* oil has a significant immunopotentiating effect in STZ-diabetic hamsters with regard to macrophage phagocytic activity. These results are in accordance with previous findings (Swamy and Tan, 2000; Haq et al., 1999), in which *N. sativa* exerted a stimulatory effect on macrophages through interleukin (IL)-3, which was secreted by T-lymphocytes under the effect of *N. sativa* oil. The oil was also found to increase the percentage of CD4-positive subset of T-lymphocytes (T helper [Th]₂ subset that secrete IL-3), which activates macrophages and regulates their activity (Szejda et al., 1984). Production of IL-1 β in a medium containing macrophages and *N. sativa* oil was also observed, which confirms that *N. sativa* oil has a stimulatory effect on macrophages either directly or through IL-3 (Hanafy and Hatem, 1991; Haq et al., 1995).

Differential leukocyte counts performed in treated and untreated hamsters showed a significant increase in blood lymphocyte count after treatment with *N. sativa* oil compared with untreated diabetic hamsters. This finding confirms the stimulatory effect of *N. sativa* oil on blood lymphocytes as reported previously (Haq et al., 1999; Hedaya, 1995).

The hypoglycaemic action of *N. sativa* oil reduced the accumulation of advanced glycation end-products, which are produced through non-enzymatic glycation of proteins (Maillard reaction), as indicated by the decrease in total glycated haemoglobin. These products, which occur in high concentrations in diabetic patients, are known to be involved in reduced activity of peritoneal macrophages through exhaustion of intracellular ATP levels (Liu et al., 1999; Kirstein et al., 1990). Therefore, the hypoglycaemic effect of *N. sativa* oil apparently supports its immunopotentiating activity on peritoneal macrophages.

In conclusion, we have demonstrated in the present study that *N. sativa*, which is a natural hypoglycaemic and immunopotentiating plant, exhibits its hypoglycaemic

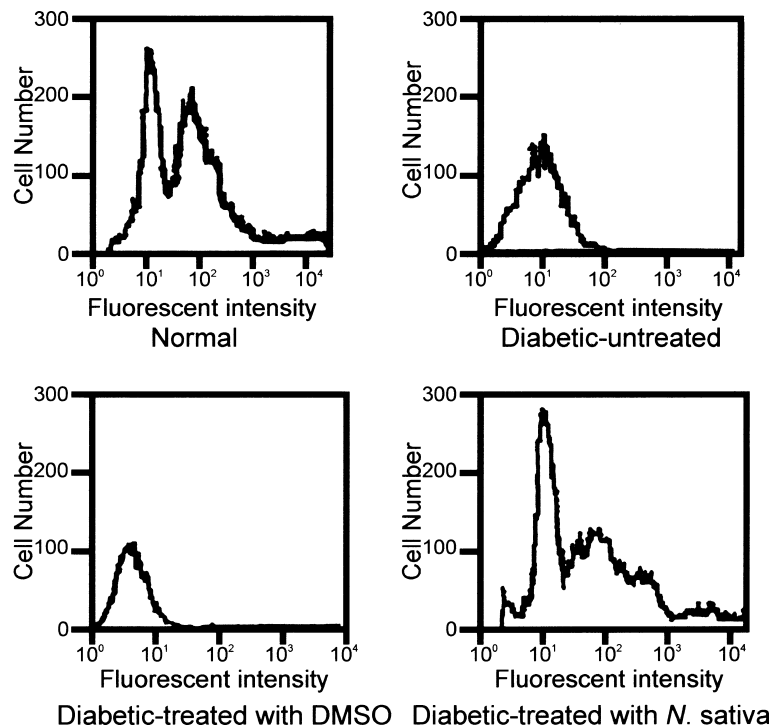


Fig. 3. Flow cytometric analysis of peritoneal macrophages in STZ-normal, diabetic-untreated, diabetic treated with DMSO and diabetic treated with *Nigella sativa* oil.

effect at least in part by decreasing liver glucose production via gluconeogenesis. *N. sativa* oil also exhibited an immunopotentiating effect on peritoneal macrophages in STZ-diabetic hamsters by increasing their phagocytic activity either directly or via stimulation of lymphocytes.

References

- Agarwal, R., Kharya, M.D., Shrivastava, R., 1979. Antimicrobial and antihelminthic activities of the essential oil of *Nigella sativa* Linn. *Ind. J. Exp. Biol.* 17, 1264–1265.
- Al-Awadi, F., Fatania, H., Shamte, U., 1991. The effect of a plant mixture on liver gluconeogenesis in streptozotocin induced diabetic rats. *Diabetes Res.* 18, 163–168.
- Al-Hader, A., Aqel, M., Hasan, Z., 1993. Hypoglycemic effect of the volatile oil of *Nigella sativa* seeds. *Int. J. Pharmacog.* 31, 96–100.
- Bassoe, C.F., Laerum, O.D., Glette, J., Hopen, G., Haneberg, B., Solberg, C.O., 1983. Simultaneous measurement of phagocytosis and phagosomal pH by flow cytometry: role of polymorphonuclear neutrophilic leukocyte granules in phagosome acidification. *Cytometry* 4, 254–262.
- Boulos, L., 1983. Medicinal plants of North Africa. Reference Publications, Algonac, MI. p. 103.
- Brownlee, M., Cerami, A., Vlassara, H., 1988. Advanced glycosylation end products in the tissues and the biochemical basis of diabetic complications. *N. Engl. J. Med.* 318, 1315–1321.
- Bunn, H.F., Haney, N., Kamin, S., Gabby, K.H., Gallop, P.M., 1976. The biosynthesis of human hemoglobin A_{1c}: slow glycosylation of hemoglobin in vivo. *J. Clin. Invest.* 57, 1652–1659.
- Carleton, C.S., Bruce, E.L., Steinkamp, J.A., 1986. In vitro and in vivo measurement of phagocytosis by flow cytometry. *Methods Enzymol.* 132, 183–193.
- Chakravarty, N., 1993. Inhibition of histamine release from mast cells by Nigellone. *Ann. Allergy* 70, 237–242.
- Class, E.J., Stewart, J., Weir, D.M., 1986. Altered immune function in alloxan-induced diabetes in mice. *Clin. Immunol.* 65, 614–621.
- Coles, E.H., 1986. *Veterinary Clinical Pathology*, fourth ed. W.B. Saunders, Philadelphia.
- Deresinski, S., 1995. Infections in the diabetic patient: strategies for clinicians. *Infect. Dis. Rep.* 1, 1–12.
- De Smet, P.A., 1997. The role of plant-derived drugs and herbal medicines in health care. *Drugs* 54, 801–840.
- Gabbay, K.H., Hasty, K., Breslow, J.L., 1977. Glycosylated hemoglobins and long term blood glucose control in diabetes mellitus. *J. Clin. Endocrinol. Metab.* 44, 859–864.
- Hanafy, M.S., Hatem, M.E., 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cummin). *Ethnopharmacology* 34, 275–278.
- Haq, A., Abdullatif, M., Lobo, P., Khabar, K.S., Sheth, K.V., Al-Sedairy, S., 1995. *Nigella sativa*: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. *Immunopharmacology* 30, 147–155.
- Haq, A., Lobo, P.I., Al-Tufail, M., Rama, N., Al-Sedairy, S., 1999. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *Int. J. Immunopharmacol.* 21, 283–295.
- Hedaya, S.A., 1995. Effect of *Nigella sativa* seed (Black seeds) extract on some haematological and biochemical parameters in rats. *Alex. J. Vet. Sci.* 2, 9599.
- Houghton, P.J., Zakara, R., Heras, B., Hoult, J.R., 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* 61, 33–36.

- Ishikawa, Y., Watanabe, K., Takeno, H., Tani, T., 1998. Effect of the novel oral antidiabetic agent HQL-975 on oral glucose and lipid metabolism in diabetic db/db mice. *Arzneim. Forsch./Drug Res.* 48, 245–250.
- Karnieli, E., Hissin, P.J., Simpson, L.A., Salons, L.B., Cushman, S.W., 1981. Possible mechanism of insulin resistance in the rat adipose cells in streptozotocin-induced diabetes mellitus. *J. Clin. Invest.* 68, 811–814.
- Kirstein, M., Brett, J., Radoff, S., Ogawa, S., Stem, D., Vlassara, H., 1990. Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet derived growth factor: role in vascular disease of diabetes and aging. *Proc. Natl. Acad. Sci. USA* 87, 9010–9014.
- Liu, B., Miyata, S., Kojima, H., Urihara, A., Kusunoki, H., Suzuki, K., Kasuga, M., 1999. Low phagocytic activity of resident peritoneal macrophages in diabetic mice. *Diabetes* 48, 2074–2082.
- Miwa, I., Okuda, J., Maeda, K., Okuda, G., 1972. Mutarotase effect on colorimetric determination of blood glucose with D-glucose oxidase. *Clin. Chem. Acta* 37, 538–540.
- Pogson, C.I., Smith, S.A., 1975. The activity of phosphoenol pyruvate carboxykinase in rat tissues. *Biochem. J.* 152, 401–408.
- Ribes, G., Sauvvaire, Y., Dacosta, C., Baccou, J.C., Lou-Batieres-mariani, M.M., 1986. Antidiabetic effects of subfractions from fenugreek seeds in diabetic dogs. *Proc. Soc. Exp. Biol. Med.* 182, 159–166.
- Riley, V., 1960. Adaptation of the orbital bleeding technique to rapid serial blood studies. *Proc. Soc. Exp. Biol. Med.* 104, 751–754.
- Swamy, S.M., Tan, B.K., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *J. Ethnopharmacol.* 70, 1–7.
- Szejda, P., Parce, J.W., Seeds, M.S., Boss, D.A., 1984. Flow cytometric quantitation of oxidative product formation by polymorphonuclear leukocytes during phagocytosis. *J. Immunol.* 133, 3303–3307.
- WHO expert committee on diabetes mellitus: second report. World Health Organ. Tech. Rep. Ser. 646, 1980, pp. 1–80.
- Zar, J.H., 1974. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ.