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Antidiabetic Properties of a Spice Plant *Nigella sativa*

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Abstract

Seeds of *Nigella sativa* (black cumin/kalonji) used in pickles as spice, have also been traditionally used in treatment of many diseases including diabetes and hypertension. Among many activities exhibited by *N. sativa* and its constituents in animal experiments, antidiabetic property is most important. Thymoquinone (TQ), a volatile oil, is one of its active constituents but antidiabetic activity has also been shown by its aqueous extract and defatted extract. *N. sativa* may be beneficial in diabetic individuals and those with glucose intolerance as it reduces appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, cholesterol, triglycerides, body weight and simulates glucose induced secretion of insulin from beta-cells in pancreas; improves glucose tolerance as efficiently as metformin; yet it has not shown significant adverse effects and has very low toxicity. In streptozotocin (STZ) induced diabetic rats it causes gradual partial regeneration of pancreatic beta-cells, increases the lowered serum insulin concentrations and decreases the elevated serum glucose. *N. sativa* has antioxidant activity and protective role of TQ against development of type I diabetes may be via NO inhibitory pathway. It also exerts an insulin-sensitizing action in hepatocytes. Seeds of *N. sativa* have been safely consumed by human patients in many clinical trials which however were not aimed to assess its antidiabetic activity. In future clinical studies may show potential of *N. sativa*, its constituents or their synthetic analogues, in prevention and control of diabetes.

Introduction

Diabetes mellitus (DM) is one of the most common lifestyle diseases. Type 2 diabetes had global prevalence estimate of 2.8% in the year 2000 and is projected to be 4.4% in 2030 [1]. Prevention and control of DM is a major challenge and requires moulding lifestyle towards more physical activity and less calorie intake avoiding sedentary habits. However most people find it difficult to change their lifestyle and look for a less cumbersome alternative. A traditional component of food that can reduce appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, body weight, and can stimulate glucose induced secretion of insulin from beta-cells in pancreas, may prove to be useful for prevention and control of diabetes mellitus. Most of these actions have been shown by seeds of *Nigella sativa* and their constituents in animal experiments and at the same time have not exhibited adverse effects. Present paper attempts to summarize the properties of *N. sativa* seeds and their constituents that may prove to be useful in prevention and treatment of diabetes.

Nigella sativa is a spice plant of family *Ranunculacea*, commonly known as black cumin or black seed. It is an erect herbaceous annual plant. It grows in Mediterranean countries and Asian countries including India, Pakistan, Indonesia, Italy and Afghanistan. In India it is called as Kalonji or kalajeera while in China it is referred as Hak Jung Chou. The seeds of *N. sativa* are used by the Indian people in pickles as spice and food preservative, while in Egypt these are used as carminative and flavouring agents in bread. Black cumin oil prepared by compressing the seeds of *N. sativa* is also used for cooking. For centuries, the seeds have been used for medicinal purpose. In old Latin it is called as 'Panacea' meaning 'cure all'. Ayurveda appreciates *N. sativa* for many qualities and bitter, warming, stimulant nature. In Ayurveda *N. sativa* has been used in wide variety of diseases like hemorrhoids, hepatitis, diarrhoea, fever, cough, and tapeworm

disease, to mention only a few of them. It is also said to enhance immunity.

Review and Discussion

The seeds of *N. sativa* contain both fixed and essential oils, proteins, alkaloids and saponin. It contains > 30% of fixed oil and 0.40 - 0.45% (w/w) of volatile oil. Eight fatty acids and thirty-two compounds have been identified in the fixed and volatile oils of *N. sativa* respectively. The main fatty acids of the fixed oil are linoleic acid, oleic acid, and palmitic acid. The major compounds of the volatile oil are thymoquinone (TQ), trans-anethole, p-cymene, alpha pinene, limonene, and carvone [2-4]. In addition to seeds, the roots and shoots of *N. sativa* have phenolic compounds like vanilic acid [5]. Much of the biological activity of the seeds has been shown to be due to thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone), whose chemical structure is $C_{10}H_{12}O_2$. Nigellone is the carbonyl polymer of TQ, isolated from *N. sativa* seeds. The polymer is far less toxic but retains much of the pharmacologic properties of TQ, which is the active principle of the plant. The pharmacological actions of the seeds of *Nigella sativa* or its constituents that have been reported include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals [6-14]. The seeds/oil have anti-inflammatory [15], analgesic [4], antipyretic [16], spasmolytic [17], bronchodialator [17], antihistaminic [18], antihypertensive [19], antitumor [20], antioxidant [18], antineoplastic [9, 21, 22], antifertility [23], antibacterial [24], antifungal [25], antiprotozoal [26], anthelmintic [27] and insecticidal [28] activities. Treatment of rats with the seed extract for up to 12 weeks has been reported to induce changes in the hemogram that include an increase in both the packed cell volume and hemoglobin [29] and a decrease in plasma concentrations of cholesterol, triglycerides and glucose [30]. The seeds of *N. sativa* are characterized by a very low degree of toxicity. Administration of either the seed extract or its oil has been shown not to induce significant adverse effects on liver or kidney functions [31]. In an investigation on LD50 of *N. sativa* seed extracts in mice, no mortality was observed with the aqueous, methanol and chloroform extracts administered orally in a dose as high as 21 g/kg. With a mega dose of 6 g/kg/day orally for 14 consecutive days, degenerative changes in hepatic cells were observed only with aqueous extract of the seeds and not with methanol and chloroform extracts [32]. This suggests a wide margin of safety. Diets with *N. sativa* fixed oils 4% or *N. sativa* essential oils 0.3% were found safe in rats as serological indices like liver and kidney functioning tests, serum protein profile, level of cardiac enzymes, electrolytes balance, red and white blood cells remained in the normal ranges even after 56 days [33]. *N. sativa* is not toxic to normal cells though TQ suppresses proliferation of tumor cells [34]. *N. sativa* may be of therapeutic benefit in diabetic individuals and those with glucose intolerance as it accentuates glucose-induced secretion of insulin besides having a negative impact on glucose absorption from the intestinal mucosa [35].

Antidiabetic activity in normal rats

In an experiment, aqueous extract of seeds of *N. sativa* was given to normal rats (10 ml/kg/day) orally for 7 to 14 days. This significantly increased serum insulin and lowered serum glucose levels without causing any evidence of histopathological damage in liver, though increase in serum glutamate pyruvic acid transaminase (SGPT) and serum gamma-glutamyl transpeptidase concentrations was observed [36]. In another experiment, oral treatment with aqueous extract of *N. sativa* for 6 weeks (2 g/kg daily) in normal rats, improved glucose tolerance as efficiently as metformin (300 mg/kg daily). It also reduced body weight without any toxic effect [37]. Le et al. gave petroleum ether extract of *N. sativa* seeds to normal rats for four weeks; it caused a 25% reduction in food intake that translated into a transient weight loss. No signs of toxicity were observed. At the end of the 4-week treatment, *N. sativa* treated rats had lower fasting plasma levels of insulin and triglycerides while fasting plasma glucose remained stable throughout *N. sativa* treatment [38]. This suggests that *N. sativa* given for first 2 weeks increases serum insulin probably by stimulating glucose induced insulin secretion from beta-cells [39] and when the therapy continues for long the serum insulin decreases as serum glucose is not enough to induce more insulin secretion. Its anorexic effect may be useful in obesity. Oil of *N. sativa* seeds (NSO) has also shown antidiabetic activity. In an experimental study, when fixed oil of *N. sativa* (1 ml/kg) was given for 12 weeks to normal rats, the serum glucose, cholesterol and triglycerides levels decreased by 16.5%, 15.5%, 22% compared to control values, respectively. In parallel, significant slowdown of the body weight evolution was observed in NSO treated animals as compared to the control group [30]. In addition to NSO, the defatted extract of *N. sativa* also increases glucose induced insulin release in isolated rat pancreatic islets in concentration-dependent manner [39].

Antidiabetic activity in STZ induced diabetic rats

Kanter and co-workers have studied effect of *N. sativa* on streptozotocin (STZ) induced diabetes in rats. Diabetes was induced in rats by a single intraperitoneal injection of STZ (50 mg/kg). The animals became diabetic within 24 hours after the administration of STZ. Intraperitoneal injection of 0.20 ml/kg volatile oil of *N. sativa* seeds for 30 days in such rats, caused gradual partial regeneration/proliferation of pancreatic beta-cells, decrease in the elevated serum glucose and increase of the lowered serum insulin concentrations [40]. Similar effects were observed in STZ induced diabetic rats

with *N. sativa* (300 - 400 mg/kg body weight) or thymoquinone (50 mg/kg body weight) once a day orally for 12 weeks [41, 42]. Similar observations were also made with crude powder of *N. sativa* (10 g/kg/day) orally for 21 days in STZ indued rats, by Khanam and Dewan [43]. In their study, the results were same with similar dose of n-hexane extract of *N. sativa*. It seems that *N. sativa* and NSO protect beta-cells against oxidative stress. STZ induces an increase in lipid peroxidation and serum nitric oxide (NO) concentrations, and decreases antioxidant enzyme activity in rats. Islet cell degeneration and weak insulin immunohistochemical staining are observed in rats with STZ-induced diabetes. Increased intensity of staining for insulin, and preservation of beta-cell numbers are apparent in the *N. sativa* treated diabetic rats. *N. sativa* treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity in STZ induced diabetes in rats [44]. Pari and Sankaranarayanan gave 20 - 80 mg/kg TQ intragastrically to STZ-nicotinamide induced diabetic rats for 45 days and observed its anti-hyperglycemic effect evidenced by decrease in glucose as well as HBA(1C) levels [45]. TQ therapy also causes renal morphologic and functional improvement after STZ-induced diabetes in rats. When TQ (50 mg/kg/day) was given for 12 weeks to STZ induced diabetic rats, three days after induction of diabetes, it reduced the glomerular size, thickening of capsular, glomerular and tubular basement membranes, increased amounts of mesangial matrix and tubular dilatation and renal function as compared with diabetics untreated [46]. During pregnancy of diabetic (STZ induced) mice, TQ treatment inhibits the rate of embryo malformations by reducing the free radicals, in addition to increasing the size and maturation of embryos [47]. Osteoporosis is a major complication in patients with diabetes mellitus, particularly in those with insulin dependency. Mechanical strength in the femur and vertebrae increases with human parathyroid hormone (hPTH) treatment. Combined treatment with *N. sativa* and hPTH is more effective than treatment with *N. sativa* or hPTH alone in improving bone mass, connectivity, and biomechanical behaviour in insulin-dependent diabetic rats with STZ-induced diabetic osteopenia [48].

Antidiabetic activity in STZ induced diabetic hamsters

NSO has insulinotropic properties in diabetes type 2-like model. Fararh et al gave NSO to STZ-induced diabetic hamsters, 6 weeks after induction of diabetes. NSO (400 mg/kg by gastric gavage) given daily 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 week of treatment, respectively. Hepatic glucose production from gluconeogenic precursors (alanine, glycerol and lactate) was significantly lower in treated hamsters indicating decreased hepatic gluconeogenesis [49]. Similar ef-

fect was observed when TQ (50 mg/kg daily) was given to STZ-induced diabetic hamsters, 4 weeks after induction of diabetes. Thirty days after TQ treatment, hepatocytes were isolated to determine liver glucose production. Glucose production after 2 h incubation of the isolated hepatocytes with gluconeogenic precursors (alanine, glycerol and lactate) was significantly lower in hamsters treated with TQ [50]. These results demonstrate that the antidiabetic action of TQ is at least partially mediated through a decrease in hepatic gluconeogenesis. In another experiment, decrease in blood glucose level together with increase in serum insulin level were observed after treatment with NSO for 4 weeks in STZ nicotinamide induced diabetic hamsters. Big areas with positive immuno-reactivity for the presence of insulin were observed in the pancreases from NSO-treated group compared to nontreated one using immunohistochemical staining [51]. This also suggests NSO helps regeneration of beta-cells and stimulates insulin secretion from them.

Antidiabetic activity in Cadmium/HAART induced diabetes

N. sativa also attenuates the damage to beta-cells of the pancreas following exposure to toxic elements such as cadmium. Cadmium chloride (0.49 mg/kg/day) injected subcutaneously, caused degeneration, necrosis, and weak degranulation in the beta-cells of the pancreatic islets in rats. Three days prior to administration of $CdCl_2$, daily intraperitoneal injection of *N. sativa* decreased the cadmium induced degeneration necrosis and degranulation in beta-cells of pancreatic islets and increased the lowered insulin levels [52]. The inclusion of HIV-1 protease inhibitors (PIs) in highly active antiretroviral therapy (HAART) has been linked to the induction of insulin resistance syndrome. Prolonged use of HAART is associated with insulin resistance in HIV-1-positive patients, which can be prevented by NSO. NSO (400 micolitres per kg body weight) has been demonstrated to prevent increases in insulin and C-peptide levels in Sprague-Dawley rats treated with a daily HAART regimen for 7 months [53]. Exposure to several different PIs, nelfinavir, saquinavir and atazanavir decrease glucose stimulated insulin secretion from rat pancreatic beta-cells. This action is mediated through increase in reactive oxygen species. Both TQ and NSO exposure increase glucose stimulated insulin secretion and ameliorate the suppressive effect of nelfinavir [54].

Possible mechanisms of antidiabetic activity

Antidiabetic activity of *N. sativa* is mediated through its multiple pharmacological actions. Rchid and co-workers have shown that the defatted extract of *N. sativa* increases glucose induced insulin release in isolated rat pancreatic islets in concentration-dependent manner [39]. In addition to stimulated insulin release from pancreas, antihyperglycemic

effect of *N. sativa* may also be mediated by extrapancreatic actions as the blood-glucose lowering effect of NSO was not paralleled by a stimulation of insulin release in the presence of NSO, nigellone or thymoquinone in STZ induced diabetic rats [55]. Nitric oxide is involved in the destruction of beta-cells during the development of type I diabetes mellitus by STZ. In STZ induced diabetes, serum and pancreatic nitrites are increased. When these rats were given TQ, serum and pancreatic nitrites decreased within three days. TQ was found to have no effect on either IkB degradation or NF-kB activation; although it significantly inhibited both p44/42 and p38 mitogen-activated protein kinases (MAPKs) which contribute to the transcriptional machinery of inducible nitric oxide synthase and NO production, respectively. These data emphasize the protective role of TQ against development of type I diabetes via NO inhibitory pathway [56]. TQ also normalises the elevated nitrites in vitro [57]. It is known to suppress expression of inducible nitric oxide synthase in rat macrophages [58]. In diabetes, *N. sativa* prevents lipid peroxidation and increases anti-oxidant defence system activity. Meral et al induced diabetes in rabbits using 150 mg/kg of 10% alloxan. Oral *N. sativa* treatment for 2 months in these rabbits, decreased the elevated glucose and malondialdehyde (MDA) concentrations, increased the lowered glutathione (GSH) and ceruloplasmin concentrations, and prevented lipid-peroxidation-induced liver damage in diabetic rabbits [59]. Anti-diabetic action of *N. sativa* is at least partly mediated through decreased liver gluconeogenesis [60]. Fararh showed that hepatic glucose production from gluconeogenic precursors (alanine, glycerol and lactate) was significantly lower in treated hamsters indicating it decreases hepatic gluconeogenesis [49]. Le et al demonstrated that in vivo treatment with petroleum ether extract of *N. sativa*, resulted in greater dose-dependent activation of MAPK p44/42erk and PKB in response to insulin in hepatocytes, which suggests that in vivo treatment with *N. sativa* extract exerts an insulin-sensitizing action by enhancing the activity of the two major intracellular signal transduction pathways of the hormone's receptor [38]. Intestinal absorption of glucose is also lowered by *N. sativa* as evidenced by the fact that the aqueous extract of *N. sativa* (0.1 pg/ml to 100 ng/ml) exerted dose-dependent inhibition of sodium-dependent glucose transport across isolated rat jejunum [37]. It appears that antidiabetic activity of *N. sativa* is mediated via multiple modes of actions [61].

Clinical studies on human beings

N. sativa has been traditionally used for treatment of diabetes and hypertension in south-eastern Morocco [62] and Jorden. A cross sectional survey of 310 diabetic patients in Jorden revealed 7.3% of them used *N. sativa* for diabetes [63]. *N. sativa* has also been safely given to human patients in some clinical trials. A double blinded, placebo controlled

experimental clinical trial was carried out in Indonesia, on adult men with central obesity which aimed to study the efficacy of *Nigella sativa* in central on serum free testosterone, body weight, waist circumference, blood sugar, lipid, uric acid, adiponectin, hs-CRP, and side effects [64]. A dose of 1.5 gm powder of Kalonji (in two capsules) was given twice a day for three months, which resulted highly significant reductions in body weight, waist circumference and systolic blood pressure, however reductions in fasting blood sugar, serum free testosterone, diastolic blood pressure, triglyceride and cholesterol-HDL, SGOT, SGPT, uric acid and hs-CRP were not significant probably due to smaller dose of *Nigella sativa* used in this trial. No side effects were detected in the treatment group [65]. *N. sativa* has been safely given to human patients in many other clinical trials, which were not aimed to asses its antidiabetic activity. In a clinical trial, oral intake of 100 and 200 mg of *N. sativa* extract twice a day by patients of mild hypertension, for 8 weeks, reduced both systolic and diastolic blood pressure; at the same time the extract caused a significant decline in the level of total and low-density lipoprotein (LDL) cholesterol [10]. In another clinical trial addition of NSO 2.5 ml twice daily to Atorvastatin 10 mg/day and Metformin 500 mg twice daily therapy resulted in significant improvement with reference to total cholesterol, low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose [65]. Single dose of 40 mg/kg of *N. sativa* or equivalent amount of its ethanolic extract was given orally to children suffering from teniasis. This reduced the percentage of fecal eggs per gram count on the days 7 and 15 and did not produce any adverse side effects [66]. Oral administration of 50% boiled extract of *N. sativa* seeds (0.375 mL/kg) to human victims of war, resulted in clinical improvement of respiratory symptoms and pulmonary function tests [67]. Smooth muscle relaxant, anticholinergic and antihistaminic effects of *N. sativa* have been found useful in patients of asthma in another clinical trial [68]. Due to its antihistaminic, antiinflamatory, antioxidant and antibacterial effects, *N. sativa* was found to be useful in patients of acute tonsillo-pharyngitis in a randomized double blind placebo controlled clinical trial [69]. In traditional medicine, *N. sativa* has been known for its anticonvulsant effects. In a double-blinded crossover clinical trial the aqueous extract of *N. sativa* (40 mg/kg/8 h) was safely given as adjuvant therapy to children with refractory epilepsy [70]. *Nigella sativa* oil was given in capsules at a dose of 40 to 80 mg/kg/day to patients of allergic diseases and no adverse reactions were reported [71]. Recently, powdered *N. sativa* seeds have been safely used in a human trial, which did not yield statistically valid conclusions in want of appropriate sample size [72]. Significant adverse or toxic effects of *N. sativa* were not observed in these studies.

It can safely be concluded that *N. sativa* seeds and NSO possess antidiabetic activity, which at least partly, is mediated by stimulated glucose induced insulin release from beta-

cells, reduced gluconeogenesis in liver, antioxidant activity and reduced glucose absorption from intestine. Animal experiments have found use of *N. sativa* seeds, NSO and its constituents including TQ, safe in appropriate doses. *N. sativa* seeds have also been used in clinical trials for diseases other than diabetes and may also be studied in safe doses, in human patients of DM, through well designed clinical trials. A word of caution will still be required if long term treatment is planned as Tennekoon and co-workers [73] have demonstrated an increase in SGPT concentrations and no significant increase in the activity of serum glutamate oxaloacetic transaminase (SGOT) in rats administered with *N. sativa* seeds. Glutamate pyruvate transaminase (GPT) is a cytoplasmic enzyme found in very high concentrations in the liver. Glutamate oxaloacetic transaminase (GOT) is present in the cytoplasm as well as in the mitochondria and is less specific than GPT as an indicator of hepatic damage since it is rapidly inactivated [74]. The oral administration of *N. sativa* also leads to marked and significant increases in gamma-glutamyl transpeptidase enzyme activity in serum after 7 and 14 days post-treatment in rats, though any histopathological evidence of hepatocellular is not observed [36]. Elevation of serum gamma-glutamyl transpeptidase concentration is generally regarded as one of the most sensitive indices of hepatic damage [75]. An increase in gamma-glutamyl transpeptidase and GPT concentrations in the absence of hepatocyte degeneration were observed following oral administration of *N. sativa* extract and this suggests that these enzymes may have been released due to hepatocellular damage caused at the molecular level. However, an enzyme-inducing effect of *N. sativa* extract cannot be ruled out and repeated or long term use of *N. sativa* may therefore not be safe. There is also a need to monitor blood counts when *N. sativa* is given for longer period as NSO (1 ml/kg) given for 12 weeks, to normal rats decreased the counts of leukocytes and platelets [30].

In recent years, large number of studies have been carried out on pharmacological actions of *N. sativa* and TQ, further pre-clinical and clinical research to assess their benefits in pre-diabetics and diabetics are needed. Attempts have also been made to isolate other active principles like nigellamines [76]. Synthetic analogues of such active principles of NSO may also be more useful than TQ and this can also be an active area of further research.

Conflict of Interests

There is no conflict of interests.

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