



Comparative Anti-Oxidative Effect of Spices in Experimentally Induced Type-II Diabetes in Rats

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ABSTRACT

Nigella sativa, *Allium sativum* and *Trigonella foenum-graecum* are common dietary spices also traditionally used in the treatment of various diseases including diabetes mellitus. Clinical research has confirmed the efficacy of several spices extract in the modulation of oxidative stress associated with diabetes mellitus (DM). The therapeutic activity of each individual spice is well documented, but their effect when combined is unknown. Polyherbalism is of current interest because polyherbal formulations enhance therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events. This study evaluated the hypoglycaemic and anti oxidative activity of aqueous extract of black cumin, garlic, fenugreek individual and its combination at different doses in STZ-NT-induced diabetic rats. Diabetic rats were treated with aqueous extract for 30 days. These extract significantly ($p < 0.05$) lowered the elevated fasting blood glucose, oxidative parameters but no effect seen in haematological indices. This oxidative stress was related to a decreased superoxide dismutase activity in diabetic rats. We suggested that black cumin, garlic, fenugreek and its combination could be used as antidiabetic complement in case of Type II diabetes mellitus.

Keywords: *Nigella sativa*, *Allium Sativum*, *Trigonella foenum-graecum*, Diabetes Mellitus and STZ-NT

Diabetes mellitus (DM) is a metabolic disorder with symptoms of polydipsia, polyphagia and polyuria. Experimental animal models with type-II DM exhibit high oxidative stress due to persistent and chronic hyperglycemia, which depletes the activity of the antioxidative defense system and thus promotes the generation of free radicals (Bajaj and Khan, 2012). The antioxidant status of a cell determines its susceptibility to oxidative damage, and is usually altered in response to oxidative stress (Halliwell and Gutteridge, 1999).

Clinical and experimental studies with nigella sativa seed extract have demonstrated many therapeutic effects, including immune-modulatory (El-Kadi and Kandil, 1987), anti-inflammatory (Houghton *et al.*, 1995), anti-diabetic (Kanter *et al.*, 2003a), anti-ulcerogenic (El-Dakhkhny *et al.*, 2002), and anti-oxidant effects (Javanbakht *et al.*, 2013). Most of these properties have been attributed

mainly to the quinone constituents of *Nigella Sativa*, of which thymoquinone is the main active ingredient of the isolated from the black seeds. Thymoquinone has been shown to possess strong antioxidant properties and to suppress the expression of inducible NO synthase in rat macrophages (Javanbakht *et al.*, 2013).

Trigonella foenum-graecum (fenugreek, locally as methi) seeds have been used as traditional medicines not only in diabetes but also in high cholesterol, inflammation and gastro-intestinal ailments (Sharma *et al.*, 1990). *Trigonella foenum-graecum* seeds have also previously been shown to have hypoglycemic and hypocholesterolemic effects on type 1 and type 2 diabetes mellitus patients and experimental diabetic animals (Xue *et al.*, 2007).

Garlic (*Allium sativum*) is a bulb-forming herb of the Lilliacae family. Several experimental and clinical

investigations have reported that garlic and its preparations have many effects include: (i) antioxidant effect, (ii) reduction of cancer risk, (iii) reduction of risk factors for cardiovascular diseases, (iv) antimicrobial effect against bacteria, viruses, fungi and parasites, (v) detoxification of foreign compounds and hepatoprotection, and (vi) decrease in platelet aggregation and atherosclerotic plaques (Colin-Gonzalez *et al.*, 2012; Bayan *et al.*, 2014).

Streptozocin (STZ) produced by *Streptomyces Achromogenes* is the most commonly used chemical in inducing experimental diabetes. The mechanism by which STZ destroys β -cells of the pancreas and induces hyperglycemia is still unclear. STZ produce cytolytic effect on pancreatic β -cell membranes and depletion of intracellular nicotinamide Adenine Dinucleotide (NAD) in islet cells. In addition, STZ has been shown to induce DNA strand breaks and methylation in pancreatic islet cells. The NO and ROS modulates oxidative damage (Van Bremen *et al.*, 2013; Ramakrishna and Jaiikhani, 2007). Antioxidants like superoxide dismutase (SOD) have been shown to protect cells against lipid peroxidation, the initial step in many pathological processes (Williams, 1984; Bray and Bettger, 1990). Reduced antioxidant levels as a result of augmented free radical production in experimental diabetes have been reported (Kanter *et al.*, 2003b).

There has been increasing interest regarding the use and role of natural antioxidants as a means of preventing oxidative damage in diabetes. So the present study was undertaken to investigate the comparative antioxidative effect of aqueous extracts *viz.* *Black cumini*, *Fenugreek*, *Garlic* and their mixture in STZ-NT induced type-II diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin and Nicotinamide were obtained from the Himedia chemical and was used for induction of the diabetes. All the other chemicals used in the study were of standard analytical grade.

Experimental animals

The study was conducted on 60 healthy adult male Wistar rats. Healthy adult rats of 10-12 weeks of age were procured

from Cadila Pharmaceutical Limited, Dholka, Gujarat, India and were maintained under standard management conditions. All the protocols as per the CPCSEA guidelines on the Care and Use of Laboratory animals were followed and approved (proposal No. VET COLL-13-2011) by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary Science and A.H. Sardarkrushinagar, Gujarat, India. All the experimental rats were kept under constant observation during entire period of study. The selected animals were under acclimatization for 7 days before grouping and dosing of animals.

All the rats were housed in polypropylene cages at Laboratory Animal House Facility in an environmentally controlled room with $22 \pm 3^\circ\text{C}$ temperature and 30-70% humidity. Light/dark cycles of 12 /12 hours were maintained throughout the experimental period. All necessary managemental practices were adopted to keep the rats free from stress. Rats were provided standard pellet diet (*M/S Pranav Agro Industries Ltd.*, Baroda, India). Pellet diets were given *ad libitum* with wholesome drinking water throughout the course of the experiment.

Experiment Design

Experiment was conducted for 30 days. The animals were divided into six groups each group contains 10 adult Wistar rat:

Group-I (Normal control animal):	Treated with vehicle (i.e. Normal saline).
Group-II (Diabetic control animal):	STZ-NT @ 45-110 mg/kg bwt intra peritoneal (ip) once
Group-III (Diabetic+ Black cumini):	STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg bwt Black cumini (oral 30 days)
Group-IV (Diabetic Fenugreek):	+ STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg Fenugreek (oral 30 days)
Group-V (Diabetic + Garlic):	STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg Garlic (oral 30 days)
Group-VI (Diabetic + Cumin, Fenugreek & Garlic):	STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg bwt mixture of cumin, fenugreek & garlic (oral 30 days)

Induction of diabetes

Diabetes was induced (Pellegrino *et al.*, 1998) by a single intraperitoneal injection of 45 mg/kg streptozotocin, 15 min after the i.p. administration of 110 mg/kg bwt of Nicotinamide in overnight fasted adult Wistar strain male rats. For this both Streptozotocin (STZ) and Nicotinamide were dissolved in normal saline. Diabetes or hyperglycemia was confirmed by the elevated glucose levels in blood and which was determined by glucometer at 72 hour and then on 7th day after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >200 mg/dl. Only rats which was found with permanent diabetes was used for the experimental study.

Aqueous Extract preparation

***Nigella sativa* (Black cumin) Seed aqueous extract**

Black cumin Seed aqueous extract was prepared by mixing 100 gm of the seed powder with 200 ml of distilled water using magnetic stirrer. The mixture was then filtered and lyophilized. The stock solution was prepared by dissolving 600 mg of lyophilized powder in 10 ml of distilled water. Concentrations of 12 and 25 mg/mL were prepared from this solution, for *in vivo* study (Kasim *et al.*, 2012).

***Trigonella foenum-graecum* (Fenugreek) Seed aqueous extract**

Fenugreek Seed aqueous extract was prepared by mixing 50gm of dried ground seed in a non-metallic jar and one liter of hot boiled distilled water were poured on it and was kept at room temperature for 5-8 hours for the preparation of an infusion. The 5% (W/V) concentration of fenugreek was used for the *in vivo* study (Farman *et al.*, 2009).

***Allium Sativum* (Garlic) aqueous extract**

Garlic aqueous extract was prepared by mixing dry garlic powder (0.6 gm) in 6 ml of distilled water and stirred for 20 min. This solution was centrifuged at 20,000 rpm for 5 min at 4°C. The supernatant was recovered and used (Jose *et al.*, 2004).

Hematology

Hematological parameters were performed with Auto

Blood Analyzer (Medonic CA 620/530 VET, Boule Medical AB, Sweden) by using impedance method.

Lipid Peroxidation

Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde (MDA) production by the method of Shafiq-U-Rehman (1984). The underlying principle is the reaction between thiobarbituric acid with MDA, which is secondary product of lipid peroxidation formation at pH 4. Optical Density (OD) value was recorded at 532 nm by calorimeter. Which represent the extent of peroxidation. The LPO activity was expressed as nmol/mg protein.

Superoxide dismutase (SOD)

Superoxide dismutase was estimated as per the method described by Madesh and Balasubramanian (1998). It involves generation of Superoxide by pyragallol autoxidation and the inhibition of Superoxide-dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-yl) 2,5 diphenyltetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. The color evolved is stable for many hours and is expressed as SOD Units [1 Unit of SOD is the amount (μ g) of haemoglobin required to inhibit the MTT reduction by 50%].

STATISTICAL ANALYSIS

The statistical analysis of data generated on various parameters was subjected to statistical analysis using completely randomized design (Snedecor and Cochran, 1980) and using CD values compared the treatment means. Since, the CD permits comparison of two consecutive treatment mean after arranging treatment mean in ascending or descending order, it had been thought worthwhile to compare treatment mean with all other treatment mean.

RESULTS AND DISCUSSION

The effect of aqueous spices extract and their mixture on hyperglycemia was measured using fasting blood glucose. Diabetic control rats showed severe ($p < 0.05$)

Table 1: Effect of aqueous extract of *Nigella sativa* (Black cumin), *Allium Sativum* (Garlic), *Trigonella foenum-graecum* (fenugreek or methi) and its combination on glucose, Lipid Peroxidase and Super Oxide Dismutase level of STZ-NT induced diabetic rats

Parameters	Control	Diabetic control	Black Cumin	Fenugreek	Garlic	Mixture
Glucose (mg/dl)	96.10 ± 1.9 ^c	280.93±7.2 ^a	169.70± 5.40 ^b	169.80±7.10 ^b	183.00±4.2 ^b	177.9±5.0 ^b
LPO (nmol/ml of RBC)	4.50 ± 0.13 ^d	7.70± 0.31 ^a	5.40 ± 0.22 ^b	5.80± 0.40 ^b	4.60± 0.10 ^{cd}	5.30±0.24 ^{bc}
SOD (U)	12.00± 0.46 ^a	5.40± 0.25 ^c	8.10± 0.37 ^{cd}	7.12± 0.39 ^d	10.70±0.46 ^b	9.00± 0.35 ^c

Values are expressed as (Mean ± SE) of ten animals in each group; Values bearing different superscript in small letter differ significantly in row respectively (p<0.05).

hyperglycemia compared to normal control rats (Table 1). This increased blood glucose level was significantly reduced by the treatment with aqueous extracts from group III, IV, V & VI in STZ-NT induced diabetic rats. The hypoglycemic action of the spice extract may be attributed to improved insulin sensitivity or inhibition of endogenous glucose production (Kuroda *et al.*, 2003). In addition, the active constituents in the spice mixture, i.e. alkaloids, tannins, polyphenols and other antioxidants, which are known to be bioactive antidiabetic principles, modulate various metabolic cascades which directly or indirectly lower the level of glucose in the system (Ogunola *et al.*, 2014). This result agrees with previous reports by Srivastava *et al.* (2012) and Petchi *et al.* (2014) on the antidiabetic activity of polyherbal formulations on induced diabetic rats.

The black cumin treatment for 30 days after inducing Diabetes Mellitus decreased the elevated lipid peroxide level to normal level (Meral *et al.*, 2001). With fenugreek and garlic extracts treatment to diabetic rats, there was decrease level of LPO and increase SOD level as compared to diabetic rats.

Oxidative stress refers to an imbalance between the intracellular production of free radicals and the cellular defense mechanisms. Proteins, lipids, and DNA are sensitive targets of reactive oxygen species (ROS). An excess availability of free radicals accompanied by a reduction in the capacity of the natural antioxidant systems leads to cellular dysfunction and death (Albers and Beal, 2000). Although the beta-cell cytotoxic action of STZ is thought to be mediated by the inhibition of free radical scavenger-enzymes, which enhances the production of superoxide radicals. The latter have been implicated in lipid oxidation (LPO), DNA damage, and sulfhydryl oxidation. STZ induced diabetes is associated with the generation of

ROS, which causes oxidative damage (Mohamed *et al.*, 1999). Lipid peroxidation (LPO) may bring about protein damage and inactivation of membrane-bound enzymes either through direct attack by free radicals or through chemical modification by its end products, MDA and 4-hydroxynonenal (Halliwell and Gutteridge, 1999).

In this study, erythrocyte LPO concentrations were significantly increased in the diabetic group with a reduction in the antioxidant enzyme activities of SOD in STZ-NT induced diabetic rats. However, treatment with black cumin decreased the elevated LPO and also increased the reduced antioxidant enzyme activities. The result is in consistent with the results of Houghton *et al.* (1995); Meral *et al.* (2001) and Kanter *et al.* (2004) reported an increase in lipid peroxides and a decrease in antioxidant enzymes in diabetes mellitus. Schettler *et al.* (1994) suggested that the reduced antioxidant production was due to increased oxygen metabolites causing a decrease in the activity of the antioxidant defense system. It has also been suggested by Kennedy and Baynes, (1984), that decreased antioxidant enzyme activity in diabetes mellitus is due to non-enzymatic glycosylation of the enzymes. Hence, in addition to its antidiabetic property, black cumin may have antioxidant properties that will be useful for therapeutic purposes. The results of the present study indicate that the preventive effects of black cumin may be due to inhibition of lipid peroxidation as a result of its antioxidant nature.

The results of this study demonstrated that there was increase production of Lipid peroxidase during experimental diabetes. The increase in lipid peroxidation and decrease in SOD levels in the blood are in agreement with similar findings in blood and other tissues in earlier studies (Sayed *et al.*, 2012). Lipid peroxidation may bring about protein damage and inactivation of membrane bound enzymes either through direct attack by free radicals

Table 2: Effect of aqueous extract of *Nigella sativa* (Black cumin), *Allium Sativum* (Garlic), *Trigonella foenum-graecum* (fenugreek or methi), and its combination on hematological parameters of STZ-NT induced diabetic rats

Parameters	Control	Diabetic Control	Black Cumin	Fenugreek	Garlic	Mixture
RBC (x10 ⁶ /cumm)	7.70 ± 0.25	7.40± 0.24	7.30 ± 0.21	7.50 ± 0.11	7.70± 0.24	7.50± 0.21
Hb (gm %)	13.60± 0.20	13.40±0.15	12.80± 0.39	12.90± 0.25	13.30±0.33	12.60±0.29
WBC (x10 ³ /cumm)	13.30± 1.30	12.90±1.81	14.50± 0.49	13.50± 0.80	13.50±0.60	14.40± 0.68
HCT (%)	41.50± 0.89	42.80± 0.45	40.85± 0.89	40.30± 0.53	41.70±0.71	41.20± 0.70
MCV (fl)	54.30±2.32	58.10± 2.13	56.60±2.28	54.10± 0.67	53.30±1.46	52.80± 1.42
MCH (pg)	17.80±0.61	18.30± 0.79	17.60± 0.49	17.40± 0.47	16.90±0.63	16.20± 0.75
MCHC (%)	33.00± 0.68	31.40± 0.33	31.50± 1.36	31.20± 0.70	32.20±0.47	33.20± 1.03
Neutrophils (%)	17.10± 0.83	17.70± 1.54	20.05± 0.93	18.20± 1.15	17.10±0.89	17.50± 1.09
Lymphocyte (%)	73.90± 1.34	73.52± 1.99	71.90±1.27	73.10± 1.60	73.20±1.27	71.20± 1.32
Eosinophils (%)	7.25± 0.52	7.19±0.38	6.30± 0.68	7.00 ± 0.80	7.50± 0.43	7.80± 0.65
Monocytes (%)	2.10± 0.54	1.56±0.30	1.75 ±0.32	1.60± 0.27	2.30± 0.47	3.40± 0.88

Values are expressed as (Mean ± SE) of ten animals in each group; Values bearing different superscript in small letter differ significantly in row respectively (p<0.05).

or through chemical modification by its end products, malondialdehyde and 4-hydroxynonenal (Halliwell and Gutteridge, 1999). Glucose is known to induce lipid peroxidation through activation of the lipoxygenase enzymes (Rajeswari *et al.*, 1991).

The recent investigation shows that Fenugreek seed powder tends to bring LPO level back to near normal. Increased lipid peroxidation under diabetic condition can be due to the increased oxidative stress in the cells as a result of depletion of antioxidants scavenger systems as reported by (Anuradha and Selvam, 1993). It is also shown that supplementation of *T. foenum-graecum* seeds in the diet enhances the antioxidant potential in control and in diabetic rats (Anuradha and Ravikumar, 2001). The antioxidant potential of Fenugreek seed powder which may involve some mechanism related to ROS scavenging activity. Fenugreek seed powder has a hypoglycaemic effect (Sharma, 1986), which is a necessary and sufficient requirement for the control of the complications arising from glycation and glycooxidation of proteins and membranes. Fenugreek seeds may have possible antioxidant properties as a result of reducing blood glucose levels and play a crucial role in the defence against oxygen free radicals (Sudharani *et al.*, 2012).

In this experiment garlic oil treatment generally normalizes oxidative stress in streptozotocin diabetic

rats as observed by Hfaiedh *et al.* (2011) and Madkor *et al.* (2011). In this study the SOD (antioxidant) level was improved by garlic and these findings were correlated with the findings of Ashour *et al.* (2011). The antioxidant potential of garlic oil is attributed to the presence the primary sulfur-containing compound of intact garlic bulb is γ -glutamyl cysteine which can be hydrolyzed and oxidized to form alliin (Amagase *et al.*, 2001). Alliin is converted to odoriferous thiosulfinate allicin by alliinase after crushing, cutting, chewing or dehydration during processing. During extraction with water or ageing, γ -glutamyl cysteine is converted to *S-allylcysteine* (SAC), a safe compound which contributes heavily to the health benefits of garlic (Amagase *et al.*, 2001). Previously, it was been reported that the total polyphenol content of aged black garlic was increased (10.00 mg/g), although the content of polyphenol compounds of garlic was not high (3.67 mg/g, Jang *et al.*, 2008). Therefore, increase in SAC and polyphenol compounds could be responsible for stronger antioxidant activity garlic. In other explanation, Borek, (2001) reported that the allicin (active content of garlic) which has antioxidant effects and has the ability to stimulate Glutathione peroxidase activity and preventing the increasing in hydrogen peroxide by increasing the activities of SOD.

The hematological finding (Table 2) in treated group animals (Group-III, IV, V, VI) revealed no significant (p<

0.05) change in hemoglobin (Hb), total erythrocyte count (TEC) WBC, HCT, MCV, MCH, MCHC, Neutrophils, Lymphocyte, Eosinophils, Monocytes as compared to normal control normal or diabetic control and (Group-I & II). Evaluation of haematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal (Ashafa *et al.*, 2009). It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu *et al.*, 2007). This is because it plays a role in physiological, nutritional and pathological state of an organism (Muhammad *et al.*, 2000). In normal and diabetic control rats and treated group did not elicit any changes in the haematological parameters. This might be due to the fact that there was no destruction of matured red blood cells by the STZ-NT and aqueous extracts. The STZ-NT and aqueous extracts, therefore at the dosages administered, have no deleterious effect on oxygen-carrying capacity of the blood. This is because hemoglobin, a major constituent of erythrocytes, which functions in oxygen transport and is used as an index to evaluate physical condition of an animal (Suchantabud *et al.*, 2008) was not altered.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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