

Original Research Article

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Antioxidative Potential of Spices in Experimentally Induced Type-II Diabetes in Rats

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ABSTRACT

Diabetes mellitus is the most prevalent metabolic syndrome characterized by hyperglycemia due to relative deficiency of insulin. This metabolic disorder caused by multiple etiologies characterized by absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance. Sedentary life style, stress and obesity are two major epidemiological determinants of diabetes mellitus. There has been a tremendous interest in the development of alternative medicine for diabetes. The goal of the present study was to provide *in vitro* evidence for potential inhibition of *in vitro* antioxidant and antidiabetic 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 60 days. The degree of protection was evaluated using biochemical parameters such as lipid peroxidation levels and superoxide dismutase (SOD). 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 increase lipid peroxidation level and decreased superoxide dismutase activity in diabetic rats. We suggested that black cumin, garlic, fenugreek and its combination could be a protective effect and used as antidiabetic complement in case of Type II diabetes mellitus.

Keywords

Black cumin,
Garlic, Fenugreek,
Diabetes Mellitus,
Lipid Peroxidation,
SOD and STZ-NT

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Introduction

Diabetes mellitus (DM) is a heterogeneous systemic metabolic disorder characterized by hyperglycemia, hyperlipidemia, hyperaminoacidemia, and hypoinsulinaemia. It results from insufficient insulin secretion,

insulin action or both (Joseph and Jini, 2011). DM is growing rapidly worldwide and affecting all parts of the world. According to world health organization the diabetic population is likely to increase up to 300 million or more by the year 2025 (Sy *et al.*, 2005).

The majority of cases of DM fall into two broad etiopathogenic categories. In type 1 diabetes, the cause is an absolute deficiency of insulin secretion from pancreas. In other, much more prevalent category is type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response. This endocrine disorder has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Patel *et al.*, 2011).

The number of people with diabetes is increasing day by day the main cause of this problem is urbanization, aging, physical inactivity and increasing prevalence of obesity. Present available therapies for DM include insulin and various oral antidiabetic agents such as, biguanides, sulfonylureas, glinides and α -glucosidase inhibitors. These products are expensive and many of them have a number of serious adverse effects. Therefore, the search for more effective and safer hypoglycemic agents is one of the most important areas of investigation (Upendra *et al.*, 2010).

In DM, hyperglycemia generates reactive oxygen species (ROS), which in turn cause lipid peroxidation and cell membrane damage and these free radicals play an important role in the production of secondary complications in diabetes mellitus (kidney, eye, blood vessel nerve and other vital organ damage). Thus, antioxidants have been shown to prevent the destruction of β - cells by inhibiting the peroxidation and they may provide protection against the development of diabetes (Piyush *et al.*, 2006).

Spices are very common in use in our day to day life. Either as a nutrient, to enhance taste or as a source of food these spices are being

consumed by the patient as well as healthy person. Easy availability, raw consumption, least side effects and low cost make available for therapies. Spices contain natural antioxidants (alkaloids, flavonoids, tannins, polyphenols, vitamins C and E, etc.) that can preserve β - cell function and prevent diabetes induced ROS formation (Grover *et al.*, 2002). The present study was focuses on the effect of different spices like Black cumin, Fenugreek, Garlic and their mixture on hypoglycaemic and antioxidative effect in STZ-NT induced type-II diabetic rats.

Materials and Methods

Chemicals

Streptozotocin and Nicotinamide were obtained from the Hi-media 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±3°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 60 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 cumin): STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg bwt Black cumin (oral 60 days)

Group-IV (Diabetic+ Fenugreek): STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg Fenugreek (oral 60 days)

Group-V (Diabetic+ Garlic): STZ-NT @ 45-110 mg/kg bwt (ip once) + 500 mg/kg Garlic (oral 60 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 60 days)

Induction of diabetes

Diabetes was induced (Pellegrino *et al.*, 1998) by a single intraperitoneal injection of 45 mg/kg bwt 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 50 gm 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 and oxidative stress analysis

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-xl) 2,5diphenyltetrazolium 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 (μg) 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, 1989) 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 ($p < 0.05$) 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). The active constituents in the spice like 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 (Kuroda *et al.*, 2003). This result agrees with previous reports by Srivastava *et al.*, (2012); Petchi *et al.*, (2014) on the antidiabetic activity of polyherbal formulations on induced 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). 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 and causes oxidative damage (Mohamed *et al.*, 1999). Lipid peroxidation (LPO) may bring about protein and DNA 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 Kanter *et al.*, (2004); Abdelmeguid (2010) reported an increase in lipid peroxides and a decrease in antioxidant enzymes in diabetes mellitus. Hence, in addition to its antidiabetic property, Black cumin may have antioxidant properties that will be useful for therapeutic purposes.

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 (Pardeep *et al.*, 2011; Sayed *et al.*, 2012). Lipid peroxidation 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, malondialdehyde and 4-hydroxynonenal (Halliwell and Gutteridge, 1999).

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 Fenugreek 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, 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 defense against oxygen free radicals (Sudharani *et al.*, 2012).

In this experiment, garlic extract treatment normalizes oxidative stress in streptozotocin induced diabetic rats as observed by Hfaiedh *et al.*, (2011); Madkor *et al.*, (2011). The SOD (antioxidant) level was improved by garlic and these findings were correlated with the findings of Saravanan and Ponmurugan (2010); 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, 2006). Therefore, increase in SAC and polyphenol compounds could be responsible for stronger antioxidant activity garlic. In other explanation, it was 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 (Borek, 2001).

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 (Muhammad *et al.*, 2004; 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.

Table.1 Effect of aqueous extract of black cumin, garlic, fenugreek and its combination on glucose, lipid peroxidase and super oxide dismutase level in control, diabetic and aqueous extracts treated diabetic groups during study

Parameters	Control Group-I	Diabetic control Group -II	Black Cumin Group -III	Fenugreek Group -IV	Garlic Group -V	Mixture Group-VI
Glucose (mg/dl)	106.10 ±4.00 ^d	290.20 ±7.40 ^a	175.40 ±7.70 ^c	190.20 ±4.60 ^{bc}	199.10 ±7.00 ^b	193.40 ±5.00 ^b
LPO (nmol/ml of RBC)	5.00 ±0.31 ^{bc}	10.10 ±0.55 ^a	5.80 ±0.42 ^b	6.10 ±0.52 ^b	4.60 ±0.28 ^c	5.80 ±0.29 ^b
Super Oxide Dismutase (U)	12.60 ±0.35 ^a	4.70 ±0.30 ^e	8.50 ±0.35 ^c	7.20 ±0.30 ^d	10.80 ±0.37 ^b	9.80 ±0.45 ^b

Values are expressed as (Mean±SE) of ten animals in each group.

Values bearing different superscript in small letter differ significantly in row respectively ($p < 0.05$)

Table.2 Effect of aqueous extract of black cumin, garlic, fenugreek and its combination on hematological parameters in control, diabetic and aqueous extracts treated diabetic groups during study

Parameters	Control Group-I	Diabetic Control Group -II	Black Cumin Group -III	Fenugreek Group -IV	Garlic Group -V	Mixture Group-VI
RBC (x10 ⁶ /cumm)	8.40 ±0.12	8.30 ±0.10	8.10 ±0.12	8.00 ±0.17	8.00 ±0.14	7.90 ±0.12
Haemoglobin/Hb (gm %)	13.40 ±0.35	13.60 ±0.42	13.80 ±0.29	13.20 ±0.38	13.40 ±0.34	14.10 ±0.30
WBC (x10 ³ /cumm)	15.50 ±0.16	15.00 ±0.46	15.40 ±0.25	15.20 ±0.31	15.40 ±0.55	14.80 ±0.17
HCT (%)	39.90 ±0.88	40.70 ±1.15	41.70 ±0.92	43.50 ±0.69	40.70 ±0.90	41.60 ±0.80
MCV (fl)	47.80 ±1.38	49.20 ±1.73	51.80 ±1.34	53.20 ±1.71	51.10 ±1.80	52.80 ±1.03
MCH (pg)	16.00 ±0.50	16.40 ±0.55	17.10 ±0.34	16.40 ±0.59	16.70 ±0.66	17.80 ±0.33
MCHC (%)	33.60 ±0.37	33.50 ±0.99	33.20 ±0.51	31.10 ±0.85	32.80 ±0.78	33.80 ±0.52
Neutrophils (%)	24.30 ±0.33	22.80 ±0.31	22.90 ±0.48	23.30 ±0.56	23.50 ±0.50	22.60 ±0.46
Lymphocyte (%)	69.30 ±0.52	70.30 ±0.73	70.10 ±0.72	69.80 ±0.62	69.80 ±0.92	69.30 ±0.75
Eosinophils (%)	4.30 ±0.52	4.40 ±0.32	4.10 ±0.51	4.10 ±0.35	4.50 ±0.50	5.80 ±0.56
Monocytes (%)	2.10 ±0.31	2.60 ±0.42	2.90 ±0.31	2.90 ±0.40	2.30 ±0.45	2.40 ±0.38

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).

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