Journal of Animal Research: v.9 n.3, p. 403-410. June 2019

DOI: 10.30954/2277-940X.03.2019.3

Effect of Three Spices on Oral Glucose Tolerance and Biochemical Parameters in Experimentally Induced Type-II Diabetes: A Comparative Study

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Received: 14 March, 2019 **Revised:** 25 May, 2019 **Accepted:** 29 May, 2019

ABSTRACT

The aqueous seed extract *of black cumin, fenugreek, garlic and combination of these three extracts* were evaluated for its antidiabetic potential by estimating biochemical parameters and glucose tolerance level on normal and Streptozotocin-Nicotinamide induced diabetic rats. The rats were divided into six groups as normal, diabetic control and four aqueous extracts (black cumin, fenugreek, garlic and combination of these three) treated groups. In this experiment, the aqueous extracts were administered to STZ-NT induced diabetic rats at the doses of 500 *mg/kg* BW *P.O.* per day for 30 days. The comparative effect of extracts on oral glucose tolerance test and ALT, AST, ALP, cholesterol, BUN, Uric acid, creatinine, and glucose level were evaluated. The statistical data indicated there was a significant increase in glucose, ALT, AST, ALP, cholesterol, BUN, Uric acid and creatinine level in diabetic rats as compared to normal rats. Comparative effect of aqueous treated group indicates that black cumin and fenugreek extract treated groups had better glucose tolerance as compared to other extract treated groups in diabetic rats. Conclusively, the aqueous extracts of *black cumin, fenugreek, garlic, and their combination* had beneficial effects in producing hypoglycemic effect along with significantly alleviating the altered biochemical enzymes in STZ-NT induced diabetic rats.

Keywords: Streptozotocin, Nicotinamide, Diabetes, Black cumin, Fenugreek, Garlic

Diabetes mellitus is a metabolic disturbance that results from insufficient insulin secretion and insulin resistance with the feature of persistent hyperglycemia, which eventually results in specific complications. It is one of the most common endocrine metabolic disorders that has caused significant morbidity and mortality due to multiple tissue degeneration like retinopathy, nephropathy and neuropathy which may lead in heart attack, stroke and peripheral vascular diseases and other complications (Orasanu and Plutzky, 2009).

The oral glucose tolerance test (OGTT) is an important laboratory procedure in preclinical studies to detect the therapeutic effect of drug against diabetes. The oral glucose tolerance test (OGTT) measures the physiological capability of body to use glucose, which is the body's main source of energy. In diabetes mellitus, both humans and animals, the OGTT provides an indication for relative roles of insulin secretion from the beta cells of pancreas and insulin resistance in the progression of glucose intolerance. The impact of diet composition along with pharmacologic interventions can be tested. OGTT is real time, in vivo and whole body test which helps to identify the best treatments and possibly delay or prevent the development of Type-II diabetes.

Black cumin is the spice reported to have beneficial effects in the treatment of many diseases. *It* has many potential beneficiary effects such as an antidiabetic, immunomodulatory, anti-inflammatory, anticancer, renal and cardiovascular effects as well as many other effects like antimicrobial, antiparasitic, antiasthmatic, and antihypertensive effects (Kanter *et al.,* 2003). Moreover, the seeds of Black cumin are widely used in the treatment of various diseases like bronchitis, diarrhea, rheumatism, and skin disorders (Bamosa, 2015). The efficacy of Black cumin is due to numerous active ingredient which has been isolated from seeds and oil including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, and alpha-hederin (Randhawa and Alghamdi, 2011), as well as flavonoids (Toma *et al.,* 2015).

Fenugreek seeds contain important active compound mainly trigonelline (alkaloids) and proteins like L-tryptophan and lysine. Steroidal saponins *viz*. diosgenin, yamogenin, tigogenin, neotigogenin and mucilaginous fibres present in fenugreek are thought to account for many of the beneficial effects. From many preliminary studies, it has been suggested that the fenugreek seed powder has hypoglycaemic properties (Basch *et al.,* 2003). The antidiabetic properties of a soluble dietary fibre fraction of fenugreek in normal, type-I or type-Π diabetic rats significantly improved oral glucose tolerance (Hannan *et al.,* 2007). Fenugreek administration may increase the plasma insulin levels *in vivo*. This improvement in insulin level may be attributed by fibre part of seeds which ultimately lower blood glucose level (Ribes *et al.*, 1986). Its major free amino acid, 4-hydroxyisoleucine considered as an insulinotropic in nature isolated from seeds, increased the insulin release in glucose fed hyperglycaemic rats and humans (Sauvaire *et al.,* 1998).

Garlic has a reputation in particular because of its widespread health use worldwide as a dietary as well as a therapeutic supplement. They possess a variety of effective active ingredient that exhibit antibiotic, antioxidant, hypolipidemic, anticoagulant, hypoglycemic, as well as hypotensive activities (Thomson *et al.,* 2007). The experimental studies showed that garlic had an ability to reduce blood glucose levels in diabetic laboratory animals and increase plasma insulin in diabetic rats (Jamison, 2003). The active ingredients in garlic include such as S-allyl cysteine sulphoxide (allicin), which stimulate the insulin secretion in pancreatic β-cells in normal rats (Thomson *et al.,* 2007). Since, garlic plays an important role in reducing oxidative stress and also blood sugar in diabetes, it is suggested as a substitute to anti-diabetic chemical agents.

Treatment with available pharmaceutical drugs or by sulphonylureas and biguanides are either having undesirable side effects or expensive (Rang and Dale, 1991). The uses of these agents are limited by limited action, its pharmacokinetic properties, secondary failure rates and accompanying side effects (Baquer *et al.,* 2011). Many indigenous medicinal plants have been found to be useful to successfully manage diabetes and some of them have been tested well in experimentally induced diabetic model and their active ingredients were isolated. In this regards, the present study was undertaken with the aim to find-out which spice have more potential effect in reducing the elevated glucose level in term of OGTT and on biochemical parameters in experimentally induced diabetic rats.

MATERIALS AND METHODS

Experimental animals

Adult Male Wistar rats of 10-12 weeks of age were used in this study. Rats were procured from Cadila Pharmaceutical Limited, Dholka, Gujarat, India and were housed for one week under standard conditions (well ventilated, temperature 22±2°C, relative humidity 50-60% and 12 hr day-night cycle) for acclimatization. Rats were provided standard pellet diet (M/S Pranav Agro Industries Ltd., Gujarat, India) with wholesome drinking water throughout the experiment.

Aqueous extract preparation

Black cumin (*Nigella sativa*) seed aqueous extract

The seeds of black cumin were procured from market and dried in shed at 25˚C for one week with frequent turning over after washing several times with water. Aqueous seeds extract was prepared from fine powder by mixing 100 gm of seed powder with 200 ml of distilled water using magnetic stirrer. The mixture was then filtered and lyophilized. The lyophilized powder 600 mg was dissolved in 10 ml of distilled water for stock solution. Desired concentration was prepared from stock solution and used for study (Kasim *et al.,* 2012).

Fenugreek (*Trigonella foenum-graecum* L.) seed aqueous extract

Seed were collected from the local market and were dried for 24 hours at 37˚C in hot air oven. Fine powder prepared from dry seed, 50 gm of dried ground seed were taken 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 to prepare an infusion, which contain concentration at 5% (W/V) fenugreek (Farman *et al.,* 2009).

Garlic (*Allium Sativum*) aqueous extract

Dried fine garlic powder was weighed (0.6 gm), dissolved, and stirred with 6 ml of distilled water 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)

Induction of diabetes

For induction of type -II diabetes mellitus in adult male wistar rats, the animals were kept overnight fasting then firstly nicotinamide/NT (110 mg/kg bwt) dissolved in normal saline and administrated intraperitoneally (IP). After 15 mins the rats received an I.P. injection of STZ (45 mg/kg bwt) dissolved in citrate buffer (pH 4.5). Status of diabetes was confirmed by the measuring the glucose concentration in blood before and 72 hr after streptozotocin-NT injection. Animals which exhibit their blood glucose levels above 250 mg/dl, were considered as diabetic and used for present study (Shirwaikar *et al.,* 2005).

Experimental design

The rats were divided into six groups each consists of a minimum of 10 animals. The Group 1: Control rats (Vehicle) without any treatment, Group 2 act as Diabetic control (STZ-NT); STZ @45mg/kg i.p once + NT@110mg/kg bwt i.p once, Group 3: Diabetic rat received Black cumin 500 mg/kg bwt oral as treatment, Group 4: Diabetic rat with Fenugreek (500 mg/kg bwt) treatment, Group 5: Diabetic rat with Garlic (500 mg/kg bwt) oral, Group 6: Diabetic rat + Mixture all of above spices (500mg/kg bwt). Extracts treatment will be given for 30 days

Oral glucose tolerance test (OGTT)

Evaluation the effect of aqueous extract of *black cumin, fenugreek, garlic, and their mixture* on oral glucose tolerance test (OGTT), at the end of the 30th day, 2 hrs after the last dose of the extracts, blood samples were withdrawn from tail vein of overnight fasting rats and blood glucose level was determined, indicating zero time of the test. Glucose solution (50%) in a dose of 2 gm/kg bwt was given orally (Bonner-Weir, 1988). Blood samples were obtained from the tail vein and glucose concentration determined at 0, 30, 60, 90, and 120 min after glucose loading by using a glucometer.

Biochemical Parameters

For biochemical estimation (30 days), rats were fasted overnight, and blood samples were drawn from heart under light ether anesthesia. Serum glucose, insulin, total cholesterol, triacylglycerol, urea, uric acid, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined analyzed by using Mercks Kits (*Mercks Specialities Private Ltd.,* Mumbai, India) by Clinical Analyzer (*Systronics*, Ahmedabad, India).

RESULTS AND DISCUSSION

Comparative effect of aqueous extracts on oral glucose tolerance test

After 16 hrs of fasting, the blood glucose level was still significantly ($p < 0.05$) higher in STZ-NA treated diabetic group (II). After providing oral glucose @ 2gm/kg bwt, there was an increase in blood glucose in all the groups including group-I, but after 120 minutes, the glucose clearance from blood found in all extract treated group and it reached toward its earlier- fasting blood glucose level.

As presented in table 1 the changes in the level of blood sugar observed during the two hours following administration of the glucose load. As can be seen, normal (non-diabetic) control animals had a little response to the glucose load as compared to control diabetic rats, indicating glucose intolerance resistance in the latter group. Treatment of diabetic animals for four weeks with the different aqueous extracts succeeded in completely restoring the OGTT response to that of non-diabetic congeners. In contrast, NSE-treated animals displayed a significant improvement in glucose tolerance, but the glyceamic response to the OGTT was intermediate

Table 1: Oral glucose tolerance test in different aqueous extracts treated groups

Time Interval	Group-I (Control)	Group-II (Diabetic Control)	Group-III (Black Cumin)	Group-IV (Fenugreek)	Group-V (Garlic)	Group-VI (Mixture)
0 Minute	95.00±2.40^{d}	294.30±7.20^{a}	170.60± 5.70^{c}	175.20± 4.30^{c}	190.10± 4.30^{b}	184.20± 5.60^{bc}
30 Minute	153.80± 7.00^{d}	359.10± 3.50^{a}	213.50± 6.80^{c}	222.80± 6.70^{bc}	241.10± 8.80^{b}	233.40± 10.40^{bc}
60 Minute	191.10± 3.10^{d}	397.10± 5.10^{a}	258.80± 5.50^{c}	277.70± 6.10^{b}	292.10± 6.40^{b}	281.50± 4.60^{b}
90 Minute	162.10± 6.90^{d}	349.40± 6.90^{a}	203.30± 8.60^{c}	226.40± 6.60^{b}	243.80± 4.50^{b}	235.40±7.40^{b}
120 Minute	113.80± 3.70^{e}	326.50± 7.60^{a}	176.80± 2.80^{d}	188.20± 3.40^{cd}	204.30± 2.80^{b}	199.10±5.20^{bc}

Superscripts are to be read row wise for mean comparison.

Table 2: Biochemical estimation in aqueous extracts treated diabetic rats

Parameter	Group-I (Control)	Group-II (Diabetic Control)	Group-III (Black Cumin)	Group-IV (Fenugreek)	Group-V (Garlic)	Group-VI (Mixture)
ALT (IU/L)	46.50±2.20^{c}	83.70±2.90^{a}	57.20±2.70^{b}	59.10±3.70^{b}	65.50±4.20^{b}	60.80±4.80^{b}
AST (IU/L)	97.60±4.30^{c}	126.40±3.30^{a}	107.90±3.40^{bc}	111.30±4.50^{b}	109.30±3.60^{b}	102.00±3.80^{bc}
ALP (IU/L)	106.00±1.60^{bc}	142.70±3.80^{a}	102.80±3.30^{c}	110.20±3.60^{bc}	104.90±3.80^{c}	114.00±1.90^{b}
Cholesterol (mg/dl)	69.70±2.60^{b}	110.30±3.70^{a}	79.30±5.30^{b}	74.80±3.80^{b}	78.60± 4.50^{b}	79.90±3.80^{b}
Uric Acid (mg/dl)	1.30±0.20^{c}	4.30±0.40^{a}	2.50±0.40^{b}	2.30± 0.30^{bc}	2.00±0.30^{bc}	2.10±0.40^{bc}
Creatinine (mg/dl)	0.4±0.10^{b}	0.80±0.10^{a}	0.40±0.10^{b}	0.40±0.10^{b}	0.50±0.10^{b}	0.40±0.00^{b}
BUN (mg/dl)	20.60±1.30^{b}	35.80±3.80^{a}	24.70± 2.20^{b}	24.70±1.60^{b}	20.20±1.10^{b}	24.40±1.10^{b}

Superscripts are to be read row wise for mean comparison.

between normal and diabetic controls rats. The fenugreek, garlic, and combination of these three (mixture) group restored the blood glucose at somewhat level but it remained significantly higher than black cumin extract treated group.

Comparative effect of aqueous extracts on aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and cholesterol levels in diabetic rats

The results showed that ALT, AST, ALP, and cholesterol levels remained significantly higher in the diabetic control group as compared to normal and aqueous extracts treated rats. In comparative aspect for extract treatment groups, the black cumin extracts treated rats showed a maximum decrease in ALT and ALP level as compared to other treatment group but significantly not differ with other extracts treated rats. But for AST, the mixture of these extracts group (VI) exerts better effect than black cumin, fenugreek, and garlic extract group. Fenugreek extract treated diabetic rats showed the highest decrease in cholesterol level but not it was at non significant level than treatment with an extract of black cumin and garlic.

Comparative effect of aqueous extracts on creatinine, blood urea nitrogen and uric acid in diabetic rats

The administration of the black cumin, fenugreek, garlic and their mixture extract significantly decreased total cholesterol in diabetic rats compared with normal rats. The present results showed that blood urea nitrogen, uric acid, and creatinine increased in STZ-NT induced diabetic control rats when compared with normal rats. The administration of the aqueous extracts and their mixture significantly decreased BUN, uric acid, and creatinine when compared with control diabetic rats. In a comparative point of view, among extracts groups, all the aqueous extract treated group showed a non significant.

In summary, the result exhibited that the effect of back cumin, fenugreek, garlic and their mixture extract tends to bring the values of cratinine, BUN and Uric acid close to the non diabetic control rats.

STZ is well-known to produce diabetes by damaging insulin-secreting Beta-cell. In STZ- NT induced diabetic rats the activities of AST, ALT, and ALP were significantly increased as compared to normal control rats group. The increase in aminotransferases levels may be due to the hepatocellular damage caused by STZ-induced diabetes. This hepatocellular damage is basically a membrane change that is sufficient to allow passage of intracellular enzymes to the sinusoids and from there into the peripheral blood (Garella, 1997).

Treatment of the diabetic rats with black cumin oil caused a reduction in the activity of these enzymes in blood as compared to the mean values of control diabetic rats and consequently may alleviate liver damage caused by STZ-NT. Our findings in this regards are in agreement with those reported by earlier workers (Al-Logmani and Zari, 2009; Ghlissi *et al.,* 2012). A possible explanation for the differential effects of black cumin oil on the activities of AST, ALT, and ALP in blood is that these treatments may inhibit the liver damage induced by streptozotocin (Al-Logmani and Zari, 2009).

Diabetic rats treated with fenugreek have shown significant decrease in plasma AST, ALT and ALP activity. These findings were well-supported by Khaled *et al*. (2010)and Ramesh *et al*. (2010). The ameliorative effect of fenugreek seeds has been attributed to the fibre part (4-hydroxy isoleucin) which also helps to reduce glucose level (Kadam *et al.,* 2013).

Treatment of the STZ-NT induced diabetic rats with the aqueous extract of garlic caused a reduction in the activity of these liver-specific enzymes as compared to the mean values of the diabetic group and this is in agreement with that of Sheweita *et al*. (2001). It has been also found that treatment of the diabetic rats with S-allyl cysteine (SAC- an active ingredient of garlic) caused a reduction in the activity of these enzymes in serum as compared to the mean values of the diabetic group, and consequently alleviate liver damage caused by STZ-induced diabetes (El-Demerdash *et al.,* 2005).

In the present study, the increase in cholesterol in diabetic rats could be due to increased cholesterogenesis (Kwong *et al.,* 1991). The significant reduction in cholesterol in black cumin extract treated rat as compared to control diabetic rats is in agreement with other experimental studies (Al-Logmani and Zari, 2009). The antilipidemic action of black cumin oil may reside in their ability to stimulate insulin secretion and action (Fararh *et al.,* 2002).

Treatment with fenugreek extracts leads to a significant decrease in cholesterol level as compared to control diabetic rats. This reduction may be attributed to decreased production and increased clearance of the major transporters of endogenously synthesized cholesterol. These data are in agreement with the studies reported by Riyad *et al*. (1988); Singh *et al*. (2010); Abdalla *et al*. (2012). The administration of garlic extract significantly decreased cholesterol in diabetic rats. Incontinence with the present data, other workers have reported that administration of fresh garlic or etheric garlic extracts was shown to improved lipid profile including reduction of serum cholesterol levels (Knipschild and Ter-Riet, 1989). Short-term experiments using primary hepatocyte cultures, which have proved useful as tools for screening the anticholesterogenic properties of garlic, also confirmed the cholesterol lowering effect of garlic (Yeh and Yeh, 1994). With respect to the cholesterol-lowering property of aqueous extract of garlic, it has been suggested that few constituents of garlic (hydroxy methyl glutaryl CoA reductase) may act as inhibitors for some enzymes, which induce participation of cholesterol synthesis (Gebhardt and Beck, 1996).

Our data showed that creatinine, urea, and uric acid levels increased in STZ-NT induced diabetic rats. The elevated level of aforesaid non protein nitrogen (NPN) may be due to a metabolic disturbance in diabetes mellitus and high activities of lipid peroxidation (LPO), xanthine oxidase and increased cholesterol and triacylglycerol levels (Anwar and Meki, 2003). Moreover, the main source of uric acid is from the release of purine which may be from protein glycation in diabetic muscle wasting (Anwar and Meki, 2003). Our data showed that treatment with fenugreek aqueous extract decreased the creatinine, blood urea nitrogen and uric acid levels in diabetic rats. The elevation of the creatinine and urea are due to impairment of kidney function in diabetic hyperglycemia (Almadal and Vilstrup, 1988). The significant rise in urea concentration in diabetic rats may be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination takes place that consequently leads to the formation of a large amount of ammonia which is eventually converted to urea. The breakdown of protein unit during gluconeogenesis in the liver results in increased production of urea, fostering negative nitrogen balance (Ganong, 2003). The treatment with black cumin extract leads to a significant decrease in the levels of creatinine, blood urea and uric acid in STZ-NA induced diabetic rats compared to untreated diabetic control rats. Similar findings were also reported by other researchers (Al-Logmani and Zari, 2009). The main effect of the aqueous extract of black cumin may be due to its ability to increase insulin secretion (Al-Logmani and Zari, 2009). The other possible mechanism explained by Sayed–Ahmed and Nagi (2007), suggested that thymoquinone (an active ingredient of black cumin) supplementation prevents the development of renal failure by a mechanism related, at least in part, to its ability to decrease oxidative stress and to preserve the activity of the antioxidant enzymes, as well as, its ability to prevent the energy decline in kidney tissue.

The oral administration of garlic to diabetic rats significantly decreased the altered levels of blood urea nitrogen suggesting a therapeutic role for garlic extract in protein metabolism. In the present study, the increased levels of uric acid observed in diabetic rats were restored to near normal by the administration of garlic aqueous extract indicating the free radical scavenging activity of garlic extract (Hfaiedh, *et al.,* 2013). Creatinine values also depend on the ability of the kidney to excrete creatinine. Both creatinine and blood urea nitrogen elevation are generally simultaneous. By oral treatment with aqueous extract of garlic for 30 days significantly reduced the creatinine level. These finding are well-supported by Kemmak *et al*. (2009).

The diabetic rats with Black cumin treatment lead in a reduction of elevated glucose level after 120 min. Evaluating the effect of *N. sativa* oil on gluconeogenesis and liver glucose production helps to clarify part of this hypoglycaemic mechanism since hepatic glucose production occurs through gluconeogenesis, and is known to contribute significantly to hyperglycaemia in diabetic patients (Ishikawa *et al.,* 1998). This significant hypoglycemic effect black cumin aqueous extract in diabetic rats is at least partially mediated through a decrease in hepatic gluconeogenesis (Al-Awadi *et al.,* 1991).

Fenugreek seeds have been shown to lower blood glucose levels close to normal values in various experimental animal models (Vats *et al.,* 2003). The actual ingredient responsible and mechanism by which it exerts these effects is not completely understood. However, several studies have shown that the presence of alkaloids, steroid saponins compounds, diasgenin, and trigonelline inhibit intestinal glucose uptake in vitro (Al-Habori *et al.,* 2001). An amino acid from fenugreek seed viz. 4-Hydroxyisoleucine displayed an insulinotropic property in vitro. It also stimulated insulin secretion in vivo and improved glucose tolerance in normal dogs and in the rat model of type-II diabetes (Sauvaire *et al.,* 1998). Besides aforesaid amino acid (4-hydroxyisoleucine), two more other amino acids arginine and trytophan are also having an antidiabetic and hypoglycemic effect. In addition, many trace elements, which are the components of these spices, have been found to possess antidiabetic effects (Mohamad *et al.,* 2004). Although various theories put forward by previous researchers have shown that the fenugreek seed works by inhibition of intestinal glycosidase (Riyad *et al.,* 1988) and insulin release.

The hypoglycaemic potency of garlic has been attributed to the sulphur compounds (di (2-propenyl disulphide and 2-propenyl propyl disulphide). The mechanism involves for hypoglycaemic action by garlic may be by direct or indirect stimulation of insulin secretion (Carson, 1987). Further, it is also suggested that these disulphide compounds have the effect of sparing insulin from -SH inactivation by reacting with endogenous thiol containing molecules such as cysteine, glutathione, and serum albumins (Augusti, 1996). By restoration of delayed insulin from existing pancreatic B-cells or by inhibition of intestinal absorption of glucose, the garlic cause significantly decreased the blood glucose level in glucose-loaded rats.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

The authors are grateful to the College Veterinary Science

& A.H, Sardarkrushinagar, Gujarat, India administration , particularly department of veterinary pathology for help and financial support. Part of this work was carried out at different faculty of the institution, which is gratefully acknowledged.

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