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Original Research Article

Sub-Chronic Comparative Study of Spices on Oral Glucose Tolerance and Biochemical Parameters in Experimentally Induced Type-Ii Diabetes in Rats

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Diabetes, Black cumin, Fenugreek, Garlic, Streptozotocin and Nicotinamide

ABSTRACT

The comparative effect 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 (STZ-NT @ 45-110 mg/kg bwt I.P. once) induced diabetic rats. For this study the rats were divided into six groups as normal, diabetic control and four aqueous extracts (black cumin, fenugreek, garlic and combination of this three) treated groups, each carries 10 male rats. The aqueous extracts were administered to STZ-NT induced diabetic rats at the dose of 500 mg/kg BW P.O. per day for 60 days. Liver and kidney related biochemical enzymes were evaluated. The result indicated that 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 the aqueous treated group indicates that black cumin and the 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 for producing hypoglycemic effect along with reducing other biochemical parameters in diabetic rats.

Introduction

Diabetes mellitus (Type 2) is the most prevalent form of diabetes seen throughout the world. Diabetes Mellitus (DM) is a serious metabolic health hazard in today's world. India, the highly populated country in the world has 65 million adults with this disorder and it is second only to China (Ramachandran *et al.,* 2014). DM results from insufficient insulin secretion and resistance with the feature of persistent hyperglycemia that leads to various complications (Orasanu and Plutzky, 2009).

The oral glucose tolerance test (OGTT) is an important procedural test in preclinical studies to detect the therapeutic effect of drug against diabetes. In diabetes mellitus, the OGTT provides an indication for relative roles of insulin secretion from the pancreatic β cells and insulin resistance in the progression of glucose intolerance. This test is a real-time, in vivo and whole body test which helps to identify the best treatments for this disorder.

Nigella sativa (NS), has been used since long eras in medical and culinary fields, which belongs to a dicotyledon plant species of the family Ranunculaceae. It has many physiological and pharmacological properties 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).

Many active ingredients have been isolated from seeds and oil which includes 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 seed have antidiabetic, hypocholesterolemic, and antioxidant effect (Basch *et al.,* 2003). Its pharmacological features have been demonstrated in diabetes mellitus research by detecting its functions on peripheral glucose utilization, insulin secretive actions, and the effect on the gum fiber in the intestines (Jin *et al.,* 2014). Fenugreek seeds contain important active compound mainly trigonelline (alkaloids) and proteins like L-tryptophan and lysine.

Garlic possesses a variety of active ingredients that shows antibiotic, antioxidant, hypolipidemic, anticoagulant, hypoglycemic, as well as hypotensive activities (Thomson *et al.,* 2007). 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 blood sugar in diabetes, it is suggested as a substitute to anti-diabetic chemical agents.

Diabetes treatment with market available drugs or by sulphonylureas and biguanides are either having undesirable side effects or expensive (Rang and Dale, 1991). Even sometimes their use shows side effects and failure action (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 has more potential effect in reducing the elevated glucose level in term of OGTT and on biochemical parameters in experimentally induced type –II diabetes in rats.

Materials and Methods

Drugs

Streptozotocin-Nicotinamide (HiMedia, India); Glucose estimation by Glucometer (Dr. Morepen Gluco One BG-03 Blood Glucose Monitor, Delhi, India) were used in this study. Other chemicals used were of analytical grade from S. D. Fine Chemicals Pvt. Ltd, Mumbai, India.

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 with wholesome drinking water throughout the experiment.

Aqueous extract preparation

Black cumin (*Nigella sativa*) seed aqueous extract

The seeds of black cumin were dried in shed for one week with frequent turning over after washing several times with water. Aqueous extract was prepared 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*) seed aqueous extract

Seed of fenugreek dried for 24 hours at 37˚C in hot air oven. Then 50 gm of powder were taken in a non-metallic jar and a 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 diabetes, 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 intraperitoneal injection of Streptozotocin (STZ)@45 mg/kg bwt). Diabetic status was confirmed by the measuring the blood glucose level before and 72 hr after STZ 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

Rats were divided into six treatments each consists of a minimum of 10 animals. The Group 1: Control rats (Vehicle)

Group 2: Diabetic control (STZ-NT); STZ @45mg/kg i.p once + NT@110mg/kg bwt i.p once

Group 3: Diabetic rat (as in group 2) + Black cumin 500 mg/kg bwt oral daily

Group 4: Diabetic rat (as in group 2) + Fenugreek 500 mg/kg bwt oral daily

Group 5: Diabetic rat (as in group 2) + Garlic 500 mg/kg bwt oral daily

Group 6: Diabetic rat (as in group 2) + Mixture all of above spices@ 500mg/kg bwt oral daily

Oral Glucose Tolerance Test (OGTT)

At the end of the 60th day, the effect of aqueous extract of black cumin, fenugreek, garlic, and their mixture evaluated for oral glucose tolerance test (OGTT), 2 hrs after the last dose of the extract. 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 to determine the glucose concentration at 0, 30, 60, 90, and 120 min after glucose loading by using a glucometer.

Biochemical parameters

For biochemical estimation (60 days), blood samples were drawn from retro orbital plexus of eye under light ether anesthesia. Plasma cholesterol, 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 spices on OGTT

The blood glucose level was still significantly ($p < 0.05$) higher in STZ-NA treated diabetic control rats (group-II) even after fasting for 16 hrs. After inducing oral glucose @ 2gm/kg bwt, there was an increase in blood glucose concentration in all the groups including Normal control rats (group-I), but after 120 minutes, the glucose clearance takes place from circulation in all extract treated groups and it reached nearly its earliest- fasting stage.

As presented in table 1, the treatment of diabetic rats for eight weeks with the different aqueous extracts succeeded in restoring the OGTT response to that of non-diabetic congeners. In contrast, *Nigella sativa* treated animals displayed a significant improvement in glucose tolerance, but the glycemic response to the OGTT was intermediate between normal and diabetic controls rats. The fenugreek, garlic, and combination of this three (mixture) group restored the blood glucose at somewhat level, but it showed significantly higher than black cumin extract treated group.

Comparative effect of spices on biochemical parameters

The results showed in table 2 indicates that ALT, AST, ALP, and Cholesterol levels remained significantly higher in the diabetic control (II) rats as compared to normal and aqueous extracts treated rats. When comparing extract treatment groups, the black cumin receiving rats exert maximum ameliorating action for ALT, AST, and ALP enzymes as compared to other treatment group but significantly not differ with other extracts treated rats. Fenugreek extract treated diabetic rats (group -IV) showed the highest decrease in cholesterol levels than other treated groups.

The administration of the aqueous extracts (0.5gm/kg bwt) and their mixture significantly decreased BUN, uric acid, and creatinine when compared with control diabetic rats. In comparative point of view, among extracts groups, *Nigella sativa* (black cumin) treated group showed extremely better ameliorative effect than others, and the increased level of these enzymes was subsided by this extract nearly to the non-diabetic control rats.

By the cytolytic effect of STZ on insulin-secreting β cells of the pancreas leads in diabetes, while Nicotinamide (NT) restore some damage β cell, thereby creating the model of type-II diabetic rats. In STZ- NT induced diabetic rats the activities of AST, ALT, and ALP were significantly increased as compared to non-diabetic control rats. This increase in aminotransferases levels may be due to the hepatocellular damage induced by STZ, which leads to leakage of intracellular enzymes to the sinusoids and from there into the peripheral circulation (Garella, 1997).

Treatment of the diabetic rats with black cumin 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. A possible explanation for these effects of black cumin on the activities of AST, ALT and ALP may be due to inhibition of liver damage induced by STZ (Al-Logmani and Zari, 2009). Our findings in this regards are in agreement with those reported by earlier researchers (Al-Logmani and Zari, 2009; Ghlissi *et al.,* 2012).

Diabetic rats treated with fenugreek have shown a significant decrease in plasma AST, ALT and ALP activity. 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). These findings were well-supported by Khaled *et al.,* (2010); Ramesh *et al.,* (2010).

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

In the present study, the increase in cholesterol in diabetic rats could be due to increased cholesterogenesis (Kwong *et al.,* 1991). The black cumin may have the ability to stimulate insulin secretion and there by inducing antilipidemic action (Fararh *et al.,* 2002). These alleviating actions for cholesterol by black cumin extract treated diabetic rats are in agreement with other experimental studies (Al-Logmani and Zari, 2009)**.**

Upon administration of fenugreek and garlic extracts leads to a significant decrease in cholesterol level as compared to control diabetic rats. This effect by fenugreek may be due 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). For garlic, the short-term experiments using primary hepatocyte cultures, which have proved useful as tools for screening the anticholesterogenic properties, also confirmed the cholesterol lowering effect of garlic (Yeh and Yeh, 1994). This cholesterol-lowering property by garlic, may be due to few active chemical constituents of garlic (hydroxy methyl glutaryl CoA reductase), which may act as inhibitors for some enzymes, which induce precipitation of cholesterol synthesis (Gebhardt and Beck, 1996). Incontinence with the present data, other researcher have reported that administration of fresh garlic or ether garlic extracts was shown to improved lipid profile including reduction of serum cholesterol levels (Knipschild and Ter-Riet, 1989).

Our data showed that creatinine, urea, and uric acid levels increased in diabetic rats. The main source of uric acid has increased the release of purine which may be from protein glycation in diabetes and muscle wasting (Anwar and Meki, 2003). In our study, the fenugreek extract treated rats showed decreased in creatinine, blood urea nitrogen (BUN) and uric acid levels in diabetic rats (Group-II). 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 and this may 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 elevated 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 nearly 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 is generally simultaneous. By oral treatment with aqueous extract of garlic for 60 days significantly reduced the creatinine level. These findings are well-supported by Kemmak *et al.*, (2009).

As presented in table 2, diabetic rats with *Nigella sativa* treatment resulted 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 by black cumin aqueous extract in diabetic rats is at least partially mediated through a decrease in hepatic gluconeogenesis (Al-Awadi *et al.*, 1991).

Table.1 Oral glucose tolerance test in control, diabetic and aqueous extracts treated diabetic groups on day 60 of experiment

Minute interval	TREATMENT- GROUPS					
	Control Group –I n =10	Diabetic control Group –II n =08	Black Cumin Group –III n =09	Fenugreek Group –IV n =08	Garlic Group –V n =08	Mixture Group –VI n =08
0 minute	106.5 ± 4.0[d]	305.0 ± 4.9[a]	182.5 ± 5.4[c]	192.2 ± 6.5[bc]	203.5 ± 6.5[b]	201.6 ± 4.1[b]
30 minute	177.2 ± 3.0[e]	364.3 ± 4.5[a]	225.0 ± 6.2[cd]	238.1 ± 3.7[bc]	248.7 ± 8.2[b]	220.1 ± 5.7[d]
60 minute	196.9 ± 7.3[e]	392.2 ± 4.2[a]	258.3 ± 5.6[b]	271.5 ± 7.4[cd]	304.6 ± 5.8[b]	286.5 ± 4.9[c]
90 minute	179.1 ± 3.2[e]	347.7 ± 12.0[a]	218.1 ± 5.7[d]	230.3± 4.4[cd]	250.2 ± 4.5[b]	246.8 ± 4.4[bc]
120 minute	127.4 ± 2.0[e]	338.6 ± 7.5[a]	168.2 ± 1.4[d]	179.8 ± 4.0[cd]	202.3 ± 7.5[b]	193.6 ± 5.9[bc]

Table.2 Biochemical estimation of various enzymes in control, diabetic and aqueous extracts treated diabetic groups on day 60 of experiment

Parameter	TREATMENT- GROUPS					
	Control Group –I n =10	Diabetic control Group –II n =08	Black Cumin Group –III n =09	Fenugreek Group –IV n =08	Garlic Group –V n =08	Mixture Group –VI n =08
ALT (IU/L)	50.5 ± 2.5^{d}	74.4 ± 4.9^{a}	53.3 ± 1.1^{cd}	62.2 ± 3.9^{bc}	62.0 ± 3.6^{bc}	65.9 ± 2.6^{ab}
AST (IU/L)	101.8 ± 1.6^{d}	138.7 ± 3.1^{a}	105.4 ± 2.5^{cd}	114.6 ± 3.8^{b}	113.3 ± 4.5^{bc}	116.7 ± 2.5^{b}
ALP (IU/L)	102.3 ± 3.3^{d}	155.5 ± 4.3^{a}	114.2 ± 3.4^{c}	120.7 ± 2.8^{bc}	125.8 ± 2.1^{b}	117.0 ± 2.7^{bc}
Chol (mg/dl)	68.8 ± 1.7^{d}	114.7 ± 5.2^{a}	83.9 ± 4.0^{b}	69.1 ± 1.0^{cd}	79.6 ± 2.7^{bc}	82.2 ± 5.4^{b}
UA (mg/dl)	0.9 ± 0.1^{c}	4.2 ± 0.7^{a}	1.1 ± 0.1^{bc}	1.9 ± 0.25^{b}	1.4 ± 0.2^{bc}	1.2 ± 0.2^{bc}
Creatinine (mg/dl)	0.4 ± 0.1^{d}	1.0 ± 0.1^{a}	0.5 ± 0.0^{cd}	0.68 ± 0.04^{bc}	0.7 ± 0.1^{b}	0.6 ± 0.1^{cd}
BUN (mg/dl)	18.9 ± 1.1^{c}	33.4 ± 1.6^{a}	22.6 ± 1.5^{c}	16.7 ± 1.2^{c}	22.6 ± 1.7^{c}	27.1 ± 2.0^{b}

- Superscripts are to be read row wise for mean comparison.
- Mean without any superscripts in row do not differ significantly ($P < 0.05$).

Fenugreek seeds have been shown the effect to lower blood glucose levels close to normal values in various experimental animal models (Vats *et al.,* 2003). The exact mechanism for the hypoglycemic effect of fenugreek is unknown. However, several studies have shown that the presence of active ingredients like alkaloids, steroid saponins compounds, diosgenin, and trigonelline inhibits 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).

The mechanism involves for hypoglycaemic action by garlic may be by direct or indirect stimulation of insulin secretion (Carson, 1987). The hypoglycaemic potency of garlic has been attributed to the sulphur compounds (di (2-propenyl) disulphide and 2-propenyl propyl disulphide). 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 in Glucose tolerance test.

In conclusion, oral administration of black cumin, fenugreek, garlic and their combination extracts in experimentally induced (STZ-NT) diabetic rats result in reduction of blood glucose level and other biochemical enzymes at significant level. Out of these three spices Nigella sativa have better effect than others in term of glucose tolerance and blood biochemical parameters.

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