RESEARCH PAPER

Control of hyperglycemia by the use of black cumin seeds (Kalunji) in diabetic mice

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Abstract

Diabetes is a fatal disease that is caused due to abnormality in insulin secretion. Herbs are considered good source for its treatment and have no harms and side effects. That's why the experiment was well designed to treat the hyperglycemia by using the Black cumin seeds (Kalunji) in diabetic mice. Mice were arranged into three groups, Group I (-ve control), Group II (+ve control) and Group III (treated) respectively. Mice kept in Group I were healthy while the Mice in Group II and Group III were injected with single dose (200 mg / kg body weight) of Alloxan monohydrate in peritoneum to induce diabetes. The recommended dose of extract of Black cumin seeds of 1000 mg / kg body weight in form of watery suspension 0.1 ml / 10 gm of body weight with a maximum of 0.3 ml was given to mice of Group III . Mice showed decrease in blood sugar level from 77.33 ± 3.53 mg / dl to 96.00 ± 3.46 mg / dl; decrease in feed intake from 3.00 ± 0.23 grams / 24 hrs to 4.13 ± 0.23 grams / 24 hrs; gain in body weight from 30.40 ± 0.84 grams to 35.53 ± 1.80 grams; decrease in level of serum urea from 10.24 ± 0.77 mg / dl to 15.6 ± 1.43 mg / dl; increase in creatinine level from 0.15 ± 0.02 mg / dl to 0.34 ± 0.74 mg / dl and uric acid from 1.23 ± 0.43 mg / dl to 1.32 ± 0.71 mg / dl at 0 and 21 days respectively. Histological examination of kidney section showed normal glomerular arrangement in Black cumin treated mice.

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Introduction

Diabetes is a type of metabolic issue that is because of abnormal state of glucose in blood. It causes steady reduction in execution of various organs of body, for example, eye, nerves, kidney, veins and heart (De Vegt *et al.*, 2001). It is because of strange emission and reaction of insulin (Patlak, 2002). Diet, age, heftiness, family ancestry, and physical inertia are chance factors that lead to type II diabetes. Red and refined meat is essential reason for dietary hazard (Bendinelli, 2012). Utilization of sugar improved drinks lead to diabetes. Utilization of organic products can diminish the danger of diabetes (Cooper *et al.*, 2012). There are available 60 hereditary variations that are worried about diabetes (Morris *et al.*, 2012). Hazard models that depend on phenotype give more noteworthy analysis that that of genotypic data. There is available physical wonder between the earth, way of life and hereditary elements that present clarification for the danger of sort II diabetes (Landenberg *et al.*, 2014).

Diabetes is one of major cause of deaths in the world. In 2012 there took place 1.5 million deaths due to diabetes. Diabetes has increased among people aged 15–69 years since 1990 (Lanzen, 1998). Western Europe, have possibility of upgrade of diabetes by 2025—a tragic wake-up Call. The number of adults with diabetes in the world increased from 108 million in 1980 to 422 million in 2014. Ratio of diabetes mellitus (DM) and impaired glucose tolerance (IGT) were calculated by a survey that was performed in the rural and urban areas of all the four provinces of Pakistan. Overall glucose intolerance (DM + IGT) was 22.04% in urban and 17.15% in rural areas (Shera *et al.*, 2006).

Herbs are utilized as prescription in Indian arrangement of drug for old time. *Nigella sativa* (NS) has a place with family Ranunculaceae. It has a ton of restorative properties. It is usually called as dark cumin (Al-Awadi *et al.*, 2009). Dark cumin seeds were utilized for treatment of diabetes and kidney disappointment in Unani framework (Alhaj *et al.*, 2009). It has been customarily used to fix distinctive infections that are identified with respiratory wellbeing, stomach and intestinal wellbeing, kidney and liver capacity, circulatory and invulnerable framework support, and for general by and large prosperity (Ahmed, 2002). Seeds of NS comprise of 26.7 % protein, 28.5% fat, 24.9% sugars, 8.4% unrefined fiber and 4.8 % fiery debris on dry weight premise (Al-Othman *et al.*, 2006). About 32 mixes have been recognized, which comprise of thymoquinone (30-48%), thymohydroquinone, dithymoquinone, p-cymene (7-15%), carvacrol (6-12%), 4 terpineol (2-7%), t-anethol (1-4%), sesquiterpene longifolene (1-8%) and á-pinene. Other follow components are carvone, limonene, and citronellol. A considerable lot of these mixes can create pharmacological impacts in people (Badary *et al.*, 1999). NS is utilized in treatment of diabetes. It jelly pancreatic beta cells and causes to diminish oxidative pressure (Fararh *et al.*, 2002). By ensuring the pancreatic B-cells, NS diminished the dimension of malonaldehyde in pancreas and nitric oxide level in serum (Gali-Muhtasib *et al.*, 2004).

The point of this investigation was to assess the viability of concentrate of Black cumin seeds for control of hyperglycemia. In addition so as to advance the portion of Black cumin seeds concentrated for treatment of diabetes.

Materials and methods

Animal model

Complete 15 grown-up pale skinned person mice of about a month old were utilized for the investigation. Mice with 24-25 g of weight were taken from the creature house, division of Zoology, GC University, Lahore. Mice were raised at room temperature of 25 °C, relative stickiness 55-60% and light dim cycle of 12h: 12h with lights on at 6.00. They are allotted in ventilated enclosures with floor region of 756 Cm². All creatures were given cardboard safe house, wooden stick and settling materials. The creatures were encouraged sustained Altromin 1319 (Brogaarden, Denmark) and faucet water. They were acclimatized for the time of multi week. Diet was given in type of business pelleted mouse feed. Sustenance admission

was around 15 g for each 100 g of body weight every day; water admission was roughly 15 ml for every 100 g of body weight every day. Sustenance pellets were comprising of 4.9% fat, 3.2% fiber and 18% protein. Gauged feed was given to the mice after time of 24hours. Mice were sustained with poultry feed of 6gm/mice.

Maintenance
The mice were kept in plastic with wire wall cages of 756 cm². Cage is made up of plastic base and wire walls. Bedding material of wooden sticks was placed at the base of cage. Appropriate ventilation was kept in the cage.

Experimental design
Nigella sativa seeds were got from general herb store close Old Anarkali Lahore. Seeds were air dried in the wake of washing and cleaning with water. Seeds were s*et al*l through night in hot stove having temperature of 42 °C. The seeds put on their consistent weight. Dark cumin seeds having weight of 15 gm were ground in 100 ml of refined water in blender until complete pounding and blending. A supply of rough suspension a couple of minutes before each test was set up by taking 10 ml from stock arrangement contain 150 ml, at that point a volume of 0.1 ml/10gm B.W with a maximum of 0.3 ml. Gathering Iu treated orally with water suspension of *Nigella sativa* seeds at a portion of (1000) mg/kg B.W dependent on our past investigation (Al-Shebani, 2005).

Alloxan Monohydrate was utilized for enlistment of diabetes. It influenced the beta cells of pancreas. Mice were famished for the duration of the night. Alloxan was arranged crisp in refined water portion of 200mg/kg was given to each starved mice. Mice were given with 10% sucrose water to evade from abrupt hyperglycemia. The mice were not influenced with hyperglycemia in the wake of giving high portion at that point given with low portion of 100mg/kg at seventh day. Blood glucose dimension of mice was analyzed at 0, seventh, fourteenth and multi day. Mice with150mg/dl glucose levels were considered as diabetic. Three gatherings of mice were made amid

analysis. Gathering I (- Ve control) having 3 mice with no diabetes, Group II (+Ve control) comprising of 6 mice with diabetes acceptance getting no treatment and Group III (treated) involving 6 mice treated with Black cumin remove for 14 days.

Ketamine was blended with the water in proportion of 1:2. The portion of 0.1 ml was infused intraperitoneally to each mouse. Blood was gathered via heart cut. Blood of 3 ml was gathered in EDTA tubes by utilizing 5cc syringes. Mice with glucose dimension of 120 mg/dl were considered as would be expected, while mice with blood glucose dimension of in excess of 150 mg/dl were considered as diabetic.

Tails of mice were punctured and Blood was gathered on segment of glucometer for estimating glucose level. Blood was permitted to cluster at 37 ° C for serum seperation. Serum was isolated in type of straw shading supernatant from the blood. Supernatant was gathered with assistance of pipette and set in eppendorf. Test was centrifuged at 5000 rpm for 15 min. After centrifugation serum was isolated in sterile cylinders and cylinders were marked and put in cooler at temperature of 4 °C. Renal capacity tests were performed to watch the adjustments in the kidney amid the investigation. Serum Urea, Serum Creatinine and Serum Uric analyses were performed by help of Rotem's RF Tester Kit. For histological examination, microtomy of expelled tissue was performed. Every single histological finding were assessed utilizing PC based picture investigations to think about the organ harm. Infinitesimal pictures were caught by utilizing a trinocular magnifying instrument (IRMECO-GmbH show IM-910, 21493 Schwarzenbk/Germany showed on a PC through an extension Tek® (scope photograph 3.0).

The information was introduced as Mean ± S.E.M. We utilized the investigation of fluctuation (ANOVA) and Tukey's different examination test as post hoc was connected for buries bunch correlation. In all cases, a p estimation of under 0.05 was viewed as noteworthy. Measurable investigation was finished by utilizing SPSS form 11.5 programming.

Results

Blood Sugar level

Anti-hyperglycemic activity of extract of Black cumin seeds was observed in the 6 Swiss albino mice with age of 4-5 weeks having 29 grams mean body weight. Single intra-peritoneal injection of 0.1ml (100 µl) of Alloxan monohydrate at dose of 1000mg /kg body weight was given to mice.

Table 1. Change in Blood sugar level, Body weight and feed intake in control and treated mice.

Days	Blood sugar level (mg/dl) (Normal: ± 8.2 mg/100 ml)			Body weight of mice in grams (Normal: 19.4±1.8) For 5 weeks			Weight of feed intake in grams/24 hrs (Normal: 4.4±0.1)		
	Normal	Diabetic	Treated	Normal	Diabetic	Treated	Normal	Diabetic	Treated
0	89.67 ± 3.48[b]	91.00 ± 2.65[b]	77.33 ± 3.53[a]	30.03 ± 0.69[a]	30.47 ± 0.55[a]	30.40 ± 0.84[a]	2.80 ± 0.12[a]	2.97 ± 0.23[a]	3.00 ± 0.23[a]
7	89.67 ± 3.48[a]	223.00 ± 7.57[b]	222.00 ± 6.24[b]	32.40 ± 0.40[a]	27.57 ± 0.38[b]	32.67 ± 0.95[b]	2.90 ± 0.06[a]	3.60 ± 0.26[b]	3.40 ± 0.10[b]
14	85.00 ± 2.89[a]	224.67 ± 6.36[c]	110.00 ± 5.77[b]	33.57 ± 0.48[a]	26.10 ± 0.78[b]	33.83 ± 1.11[b]	3.23 ± 0.09[a]	4.80 ± 0.21[c]	3.80 ± 0.15[b]
21	87.00 ± 2.89[a]	250.00 ± 3.79[c]	110.00 ± 5.77[b]	34.60 ± 0.64[a]	25.27 ± 0.78[b]	35.53 ± 1.80[b]	3.40 ± 0.12[a]	6.20 ± 0.23[c]	4.13 ± 0.23[b]

Means with different superscript differ significantly at *P* < 0.05. Similar alphabets represent statistically non significant values.

This dose causes rise in blood glucose level higher than 150 mg /dl from day 0 to 21 days. Thinning of body hair, timed eyes, sluggish body movement and some time shivering was observed in diabetic mice. There was no increase in blood sugar level from 88.67±3.48 mg /dl to 87.00±3.48 mg / dl at 0 and 21 days respectively in group I.Where as in group II the increase in blood sugar level occurred from 90.00±2.65 mg /dl to 250.00±3.79mg /dl at 0 and 21 days respectively given in Table 1.

Table 2. Change in Serum urea, Serum creatinine and Serum uric acid in control and treated mice.

Days	Serum urea level (mg/dl) Normal: Average: 5.70 Variation: 3.36-6.8			Serum creatinine level (mg/dl) Normal: Average: 0.10 Variation: 0.08-012			Serum uric acid level (mg/dl) Normal: Average: 0.04 Variation: 0.02-0.06		
	Normal	Diabetic	Treated	Normal	Diabetic	Treated	Normal	Diabetic	Treated
0	10.82 ± 0.59[a]	1.46 ± 0.74[a]	10.24 ± 0.77[a]	0.13 ± 0.37[a]	0.31 ± 0.15[a]	0.15 ± 0.02[a]	1.08 ± 0.089[a]	1.09 ± 0.10[a]	1.23 ± 0.43[a]
21	17.4 ± 1.02[a,b]	37.2 ± 9.43[b]	15.6 ± 1.43[a]	0.30 ± 0.07[a]	0.76 ± 0.09[b]	0.34 ± 0.74[a]	1.24 ± 0.019[a]	1.41 ± 0.064[a]	1.32 ± 0.71[a]

Means with different superscript differ significantly at *P* < 0.05. Similar alphabets represent statistically non significant values.

Diabetic mice treated with extract of Black cumin seeds indicated that there was decrease in blood sugar level in treated group as compared to diabetic group. With the passage of time at 21st day of treatment sugar level was reduced from 77.33±3.53 to 96.00±3.46at 0 and 21 days respectively which is toward normal as shown in Table 1.

Body Weight

There was an increase in body weight of mice from 30.03±0.69 to 34.60±0.64 grams at 0 and 21 days respectively in group I. Where as in group II the decrease in body weight occurred from 30.55±0.62 to 25.27±0.78 grams at 0 and 21 days respectively during same period of time.

There was significant decrease ($P < 0.05$) in body weight in group II as compared to group I. However mice treated with extract of Black cumin seeds showed comparable value of gain of body weight from 30.40±0.84 to 35.53±1.80 which is toward normal as shown in Table 1.

Feed Intake
There was an increase in feed intake from 2.80±0.12 grams /24 hrs to3.40±0.12 grams /24 hrs at 0 and 21 days respectively in group I. Where as in group II the increase in feed intake occurred from 2.90±0.20 gram /24 hrs to 6.18±0.21 grams /24 hrs at 0 and 21 days respectively. There was significant increase in feed intake in group II from 2.97±0.23 to 6.20±0.23 gram /24 hrs at 0 to 21 days exposure respectively.

The results indicated that there was highly significant ($P <0.05$) increase in feed intake in diabetic group II as compared to control group I. However mice treated with extract of Black cumin seeds showed comparable value of feed intake from 3.00±023 to 4.13±023 at 0 and days respectively which is toward normal given in Table 1.

Serum Urea level
Serum urea level remained from 10.82±0.59mg /dl to 17.4±1.02 mg /dl at 0 and 21 days respectively in group I. Whereas, in group II the highly significant increase ($P < 0.05$) in serum urea level occurred from 1.46±0.74 mg /dl to37.2±9.43 mg /dl at 0 and 21 days respectively. Mice treated with extract of Black cumin seeds showed value of serum urea from 10.24±0.77 mg /dl to 15.6±1.43 mg /dl at 0 and 21 days in group III which is toward normal as given in Table 2.

Serum Creatinine level
Serum creatinine level remained from 0.13±0.37 mg /dl to 0.30±0.07 mg /dl at 0 and 21 days in group I. Whereas, in group II there was significant increase ($P < 0.05$) in serum creatinine level occurred from 0.31±0.15 mg /dl to 0.76±0.09 mg /dl at 0 and 21 days respectively. Mice treated with extract of Black cumin seeds showed decrease in serum creatinine level from 0.15±0.02 to 0.34±0.74 in group III which

is toward normal as shown in Table 2.

Serum Uric Acid level
Serum uric acid level remained from 1.08±0.089 mg /dlmto1.24±0.019 mg /dl at 0 and 21 days respectively in group I. whereas in group II there is significant increase ($P < 0.05$) in serum uric acid level occurred from 1.09±0.10 mg /dl to 1.41±0.064 mg /dl at 0 and 21 days respectively. Mice treated with extract of Black cumin seeds showed decrease in serum uric acid level from1.23±0.43 to 1.32±0.71 in group III which is toward normal as shown in Table 2.

Histology of Diabetic kidney
Mice from normal group showed no abnormal changes in tissues as shown in figure 1. A small portion tissue was taken from Alloxan induced diabetic mice. Due to this chemical there were small vacuolations in kidney. Glomeruli were damaged and glomerular atrophies were appeared as shown in figure 2. Extract Black Cumin seeds with dose of 1000 mg/kg B.W were given to the treated group 3. These seeds showed positive and better results in kidney tissues with normal glomeruli as shown in figure 3.

Discussion
Artificial Diabetes Induction
In this study, single intra-peritoneal injection of alloxan monohydrate (200 mg/kg) induced diabetes in Swiss Albino mice after 7 days of injection experimentally. Banerjee and Bhattacharya (1948) reported that 150-200 mg/kg of alloxan monohydrate induced diabetes in rats. In addition 70-80 mg/kg of alloxan resulted diabetes in mice. Therefore, optimized dose of alloxan monohydrate was used for induction of diabetes in the animal models (Al Shebani, 2005).

Blood Sugar level
N. sativa extract proved effective to decrease blood sugar level. +ve control mice have sugar level from 91.00±2.65 mg / dl to 250.00±3.79 mg / dl at 0 and 21 days respectively. In treated group blood sugar level decreased from 77.33±3.53 mg / dl to 96.00±3.46 mg / dl at 0 and 21 days respectively.

Similar results were reported that extract of *N. sativa* showed hypoglycemic effect in alloxan diabetic rats (Awadi *et al.*, 1991). Oral administration of 500 mg/kg extract of *N. sativa* extract decreased blood glucose level in alloxan diabetic rats (Salama, 2011).
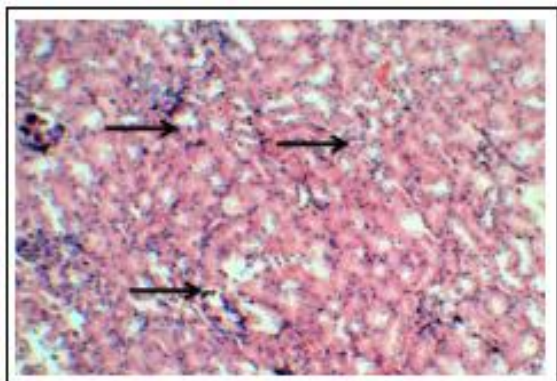

Fig. 1. Photograph of a kidney section from –ve control (group I) without any pathological abnormality. Kidney of normal mice showed normal appearance of the glomeruli (→). H & E X400.

Body Weight

The study also demonstrated that +ve control group of mice have reduced body weight from 30.47±0.55 grams to 25.27±0.78 grams at 0 and 21 days respectively.

The 1000 mg /kg body weight black cumin was given to treated group caused to gain in body weight from 30.40±0.84 grams to 35.53±1.80 grams at 0 and 21 days respectively that is toward normal. Our results showed the positive effect of 0.1ml /10gm black cumin seeds on body weight. Reduction in weight may be associated with the increased body metabolism (Nijmi *et al.*, 2013). Overweight and obesity, especially central obesity, is associated with metabolic disturbances such as diabetes and dyslipidemia (Osadebe *et al.*,, 2014).

Feed intake

Feed intake gradually reduced in mice treated with Black Cumin seeds. Similar finding were reported in albino mice treated with Black cumin seeds which reduced feed intake after 14 days treatment (Mallick *et al.*, 2007). Food intake and energy expenditure were significantly reduced with dose of 0.1ml /10gm Black cumin seeds. In +ve control group feed intake

was from 2.97±0.23 gram / 24 hrs to 6.02±0.23 grams / 24 hrs at 0 and 21 days respectively. In treated group feed intake was from 3.00±0.23 grams / 24 hrs to 4.13±0.23 grams / 24 hrs at 0 and 21 days respectively. This suggested that seeds have component to control the feed intake that is toward normal.

Serum Urea level

The present study was also designed to observe the effect of *N. sativa* on renal function by evaluation of the serum urea, serum creatinine, serum uric acid and histopathological changes of kidney. Present study revealed that in +ve control group serum urea level was from 1.46±0.74 mg / dl to 37.2±9.43 mg / dl at 0 and 21 days respectively. In treated group serum urea level was from 10.24±0.77 mg / dl to 15.6±1.43 mg / dl at 0 and 21 days respectively.

There was decrease in serum urea level that is toward normal. The normal range of urea is 10-50 mg /dl (Weber *et al.*, 2002).
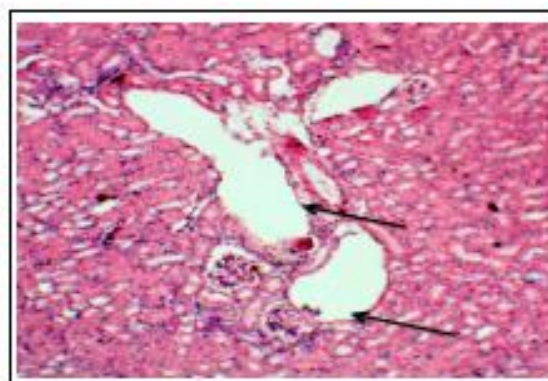

Fig. 2. Photograph of a kidney section from +ve control (group II) showing marked degeneration of the glomeruli with glomerular atrophies and severe vacuolations (→). H&E X400.

Serum Creatinine level

Present study evaluated that in positive control group serum creatinine level was from 0.31±0.15 to 0.76±0.09 at 0 and 21 days respectively. In treated group serum creatinine level was from 0.15±0.02 to 0.34±0.74 at 0 and 21 days respectively. There was decrease in serum creatinine level. The normal range of serum creatinine level is 0.5-1.4 mg /dl (Weber *et al.*, 2002).

Serum Uric Acid level

Current study demonstrated that in positive control group level of uric acid was from 1.09±0.10 mg / dl to 1.41±0.064 mg / dl at 0 and 21 days respectively. While in treated group uric acid level was from 1.23±0.43 mg / dl to 1.32±0.71 mg / dl respectively.
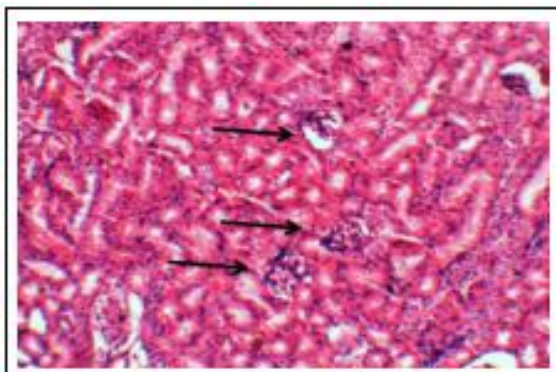

Fig. 3. Photograph of a kidney section from treated group with *Nigella sativa* showing pathological normality. Kidney of mice showed normal appearance of the glomeruli (→). H&E X400.

Histology of Diabetic kidney

Extract of Black cumin seeds proved effective as anti-diabetic. Histology of diabetic kidney has demonstrated the distorted nephrons in diabetic mice. In present Black cumin seeds treatment have prevented the destruction of nephrons and preserved architecture of kidney cells. In conclusion Black cumin seeds were effective against diabetes with a recommended dose of 1000 mg / kg body weight. Moreover histological examination of kidney depicted the prevention of structural damage to maintain their function.

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