ORIGINAL ARTICLE

# Comparative Study of Hypoglycaemic Effects of Crude *Nigella Sativa Linn* (Kalajira) and Its Ethanol Extract on STZ Induced Diabetic Rats.

Nazim Uddin[1], Zesmin Fauzia Dewan[2], Morshed Nasir[3], Moshi-uz-Zaman[4], Rekha Rani Saha[5]

**Abstract :**

*The effects of crude Kalajira and its ethanol extract (freeze dried) on serum glucose concentration in streptozotocin (STZ) induced diabetic rats were compared among 63 healthy adult rats of both sexes and the experiments were divided into three parts. Rats were divided into groups and the effects of single i.p. injection of STZ at a dose of 50 mg/ kg b.wt. on serum glucose concentration of normal rats was studied and compared with control. The effects of crude nigella at a dose of 6 gm/ kg b. wt./ day mixed with food on serum glucose concentration of STZ treated diabetic rats were studied. The serum glucose concentration in crude nigella-treated normal rats was 5.48±0.22 mmol/L and that in crude nigella-treated diabetic rats was 5.33±0.34mmol/L, significantly lower (P<0.001) when compared to STZ-treated diabetic rats (10.37±0.18 mmol/L). Later, freeze-dried ethanol extract of nigella was used and the serum glucose concentration was 4.90±0.32 mmol/L and the serum glucose concentration of those of crude nigella-treated diabetic rats was 6.87±0.31mmol/L. Here also was significant reduction of serum glucose concentration (P<0.001) compared to those of STZ control groups. The reduction of serum glucose concentration in diabetic rats following oral administration of crude nigella powder (6gm /kg b. wt. /day) mixed with food and freeze-dried ethanol extract of nigella (6gm/ kg b. wt. /day) mixed with food for 21 days were statistically significant (P<0.001) compared to STZ control but not significant (P>0.05) compared to each other suggesting that both crude powder and freeze-dried ethanol extract of nigella have similar efficacy in reducing serum glucose concentrations of STZ- induced diabetic rats. Further studies have been suggested to know the mechanism of anti-diabetic effects of nigella and to know the similar effects of various extracts of nigella upon blood sugar level of diabetic rats.*

1. Assistant Professor of Pharmacology, Moulana Bhasani Medical College, Uttara, Dhaka
2. Associate Professor of Pharmacology, Bangabandhu Sheikh Mujib Medical University, Dhaka
3. Assistant Professor of Pharmacology, Holy Family Red Crescent Medical College, Dhaka
4. Professor of Chemistry (pure), Dhaka University, Dhaka
5. Lecturer of Pharmacology, Sir Salimullah Medical College, Dhaka

## Introduction :

Diabetes mellitus is one of the major causes of disability which in its severe form will lead to loss of manpower, labour and decreased productivity in all spheres of life. Moreover, diabetes mellitus alone ranks among the top ten causes of death in the western world[1]. The true figure about persons suffering from the disease in the underdeveloped countries is lacking although there is increasing evidence that the incidence of the disease is on the

increase in the developing countries[2]. In the present world, this disease is a matter of concern because it is an important cause of morbidity and mortality throughout the world. Using the very conservative estimate of 2% diabetes in the world population of about 4.5 billion means that there are presently at least 90 million diabetics in the world[2].

Regarding treatment, a suitable drug is yet to be available which can permanently cease or cure this disease although since the discovery of insulin by Banting and Best in 1922, it is being successfully used in the treatment of diabetes mellitus. Still there are limitations to the use of insulin, which cannot be given orally. Moreover, daily injections, insulin resistance, its cost and side effects are the drawbacks of insulin. Insulin resistance is a major problem of using insulin, which may be due to abnormal insulin molecule, incomplete conversion of proinsulin to insulin, circulating insulin antagonists, defect in insulin receptors and also post-receptor defects[3]. Besides insulin, the other groups of drugs available for the treatment of diabetes mellitus are the sulfonylurea, the biguanides, the thiazolidinedione derivatives and α-glucosidase inhibitors for oral administration.

The traditionally available remedies for diabetes mellitus from natural sources in Southeast Asia may be worth mentioning. Because of local and easy availability of certain herbs, treatment of diabetes mellitus with traditional medicine would probably be cheaper if effective remedy could be provided. Herbal agents like garlic oil (*Allium sativam* Linn.), neem oil (*Melia azadirachta* Linn.), black berry seed extract (*Eugenia jambolana*), onion (*Allium cepa* Linn.) etc. are known to possess hypoglycaemic properties. Dietary intake of redgram decreased blood glucose and serum lipid profile in NIDDM patients[4]. The methanol extract of *Cajanus cajan* may contain active ingredients responsible for hypoglycaemic and hypolipidaemic effects[4]. Because they are not yet available as drugs for the treatment of diabetes mellitus, and because herbal agents with medicinal properties are available in abundance in Bangladesh, compilation of the above mentioned reports has led the present researchers to suggest that development of new drugs with lesser adverse effects could be an additional advantage to the number of presently available drugs for the treatment of diabetes mellitus.

*Nigella sativa* Linn. (kalajira) is a small pretty herb, belonging to the family Ranunculacae and grown in India, Bangladesh and the countries bordering the Mediterranean sea. The seeds of *Nigella sativa* Linn.(kalajira) are trigonous, regular, tubercular and black in colour. The seeds of *Nigella sativa* Linn. (kalajira) are used as a spicy ingredient of food in South-East Asia from ancient times. Chemical analyses of the seeds have revealed that they contain crystalline active principles such as nigellone, terpines carvone, carvene saponins, melathin and hederogenin derivative sapogenin and melanthigenin. Besides, the seeds also contain fixed and essential oils, fatty acids and protein. The fixed oil of *Nigella sativa* Linn. (kalajira) contains 0.4% dark-yellow volatile oil.

The *Nigella sativa* Linn. (kalajira) seeds have a folkloric reputation in treating several diseases. They are used by the rural people as a stimulant of CNS, as a carminative in stomach aches, as a diuretic, as immunogogue and as a galactogogue. They are excellent adjunct to purgative draughts and in toxic medicines. In eruptions of the skin the powdered seeds mixed with coconut oil are used externally in India. The rural people use it in cough, jaundice and in helminthic infestations. They are used in fever, bodyache, catarrh and, mixed with sesamum oil, applied externally in skin eruptions and on

scorpionstings. They are also added to the bread as a flavoring agent. As a galactogogue it is given to promote contraction of uterus after childbirth and for the secretion of milk. They are used in amenorrhoea and dysmenorrhoea. In large doses they are abortifacient. They are excellent adjunct to make the drugs bitter. As an antimicrobial, they are used in otitis media and sinusitis caused by both Gram positive and Gram negative organisms. Thymohydroquinone isolated from *Nigella sativa* Linn. (kalajira) seeds are used against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Sarcina lutea*, *Micrococcus lysodestikens*, and *Candida albicans*.The volatile oil of *Nigella sativa* Linn. (kalajira) is known to possess antifungal activity and is active against *Shigella sp.*, *Vibrio cholerae and E. coli*. Diethyl ether extract of *Nigella sativa* Linn. (kalajira) seeds showed antibacterial synergism with streptomycin and gentamicin and have additive antibacterial activity with streptomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and sulfamethoxazole-trimethoprim combinations. The essential oil was found to have fairly good activity against earthworms and hookworms. The hypoglycaemic effect of *Nigella sativa* oil may be mediated by extra pancreatic actions rather than by stimulated insulin release[5]. *Nigella sativa* oil has insulinotropic properties in type-II-like diabetic model[6]. *Nigella sativa* might be used in diabetic patients to prevent lipid peroxidation, increase the anti-oxidant defense system activity and also to prevent liver damage[7]. The anti-diabetic action of the plant mixture extract comprising of *nigella sativa*, myrr, gum olibanum, gum asafoetida and aloe may, at least partly, be mediated through decreased hepatic gluconeogenesis. The extract may prove to be a useful therapeutic agent in the treatment of NIDDM[8].

**Materials and method :**

The experiment was carried out in the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU) over one year period on a total of 63 adult healthy rats of Long Evans strains (Ratus Norvegicus) of either sex (excluding pregnant females), aged between three and four months and weighing between 250 and 300 gm. Rats were divided into several groups in phases as follows :

Group A (n = 9): Vehicle control

The rats were fed with normal diet and water *ad libitum* for 21 days.

Group B (n = 6): STZ-1 control

The rats were given single intraperitoneal injection of streptozotocin (50 mg/kg body weight) on the morning of Day 1 and fed with normal diet and water *ad libitum* on Day 1 and Day 2.

Group C (n = 6): STZ-2 control

The rats were given single intraperitoneal injection of streptozotocin (50 mg/kg body weight) on the morning of Day 1 and fed with normal diet and water *ad libitum* from Day 1 to Day 9.

Group D (n = 6): STZ-3 control

The rats were given single intraperitoneal injection of streptozotocin (50 mg/kg body weight) on the morning of Day 1 and fed with normal diet and water *ad libitum* for 21 days.

Group E (n = 9): Crude *Nigella sativa* control

The rats were fed with normal diet and water *ad libitum*, and were administered Crude Nigella powder (6gm/kg body weight/day) orally mixed with food from Day 1 to Day 21.

Group F (n = 9): Crude *Nigella sativa* following STZ treatment

The rats were given single intraperitoneal injection of streptozotocin (50mg/kg

body weight) on the morning of Day 1. The rats were fed with normal rat diet and

water *ad libitum* from Day 1 to Day 21. Then the rats were administered crude

Nigella powder (6gm/kg body weight/day) orally mixed with food for 21 days.

Group G (n = 9): ethanol extract of *Nigella sativa* control

The rats were fed with normal diet and water *ad libitum* and freeze dried ethanol extract of Nigella Sativa (6gm/kg. body weight/day) mixed with food for 21 days.

Group H (n = 9): Ethanol extract of *Nigella sativa* following streptozotocin treatment.

The rats were given single intraperitoneal injection of streptozotocin (50mg/kg body weight) on the morning of Day 1 and were fed with normal rat diet and water *ad libitum*. The rats were administered freeze dried ethanol extract of Nigella (6gm/kg body weight/day) orally mixed with food for 21 days.

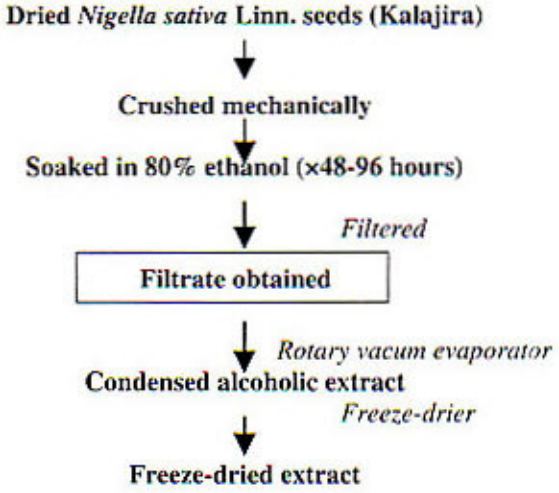


**Figure-1:** Method of obtaining the ethanol extract of kalajira seeds

**Results :**

*Effects of crude nigella powder on serum glucose concentration (mmol/L, mean ±SE) in normal and STZ induced diabetic rats:*

In the experiments, the effects on serum glucose concentration (mmol/L, mean± SE) of crude nigella powder administered orally for 21 days to normal rats (Group E) and diabetic rats (Group F) were estimated and compared with those of vehicle control (Group A) and STZ control (Group D) groups.

The serum glucose concentrations in crude nigella treated normal rats (Group E) and nigella treated diabetic rats (Group F) were 5.48 ± 0.22 and 5.33 ± 0.34 mmol/L respectively. The difference in serum glucose concentrations between crude nigella treated normal rats (Group E) and vehicle control normal rats (Group A) were not statistically significant ($P>0.05$). However, the serum glucose concentration in crude nigella treated diabetic rats (Group F) was significantly reduced ($P<0.001$) when compared to those of STZ control (Group D) diabetic rats (Fig: 2).

*Effects of freeze dried ethanol extract of nigella on serum glucose concentration (mmol/L, mean±SE) in normal and STZ induced diabetic rats:*

The effects on serum glucose concentration of freeze dried ethanol extract of nigella administered orally for 21 days to normal rats (Group G) and diabetic rats (Group H) were estimated and compared to those of vehicle control (Group A) and STZ control (Group D) groups.

The serum glucose concentration in freeze dried ethanol extract on nigella treated normal rats (Group G) and freeze dried ethanol extract of nigella treated diabetic rats (Group H) were 4.90±0.32 and 6.87±0.31 mmol/L respectively. The difference in serum glucose concentrations between freeze dried ethanol extract treated

normal rats (Group G) and vehicle control normal rats (Group A) was not statistically significant (P>0.05).

However, the serum glucose concentration in freeze dried ethanol extract of nigella treated diabetic rats (Group H) was significantly reduced (P<0.001) when compared to those of the STZ control (Group D) diabetic rats (Fig: 2).

The reduction of serum glucose concentration in diabetic rats following oral administration of crude nigella powder and freeze dried ethanol extract of nigella for 21 days was not statistically significant (P>0.05) compared to each other, suggesting that both crude powder and freeze dried ethanol extract of nigella have similar efficacy.

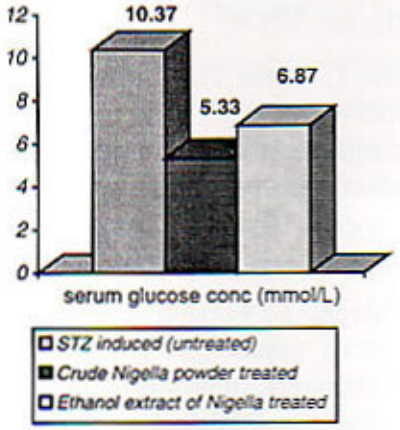


**Figure 2:** Serum glucose concentrations (mmol/L) in streptozotocin induced diabetic rats

**Discussion :**

Streptozotocin is an antibiotic extracted from *Streptomyces acromogenes*. This drug is an experimental one, which is well known for its lytic action upon the β-cells of the islets of pancreas. Chemically, streptozotocin is consist of 2-deoxy-D-glucose with a N-nitrosomethyl-urea side chain at the second carbon atom and it causes selective destruction of pancreatic β-cells in several species. The detailed mechanisms for the streptozotocin-induced cell injury is so far not clear[9]. Thus the contents of β-cells i.e., insulin, is depleted from the cells and later on, metabolized by the enzyme insulinase. The glucose in the blood therefore can not be acted upon by insulin and thus increased blood sugar level prevails. Therefore, although streptozotocin is an antibiotic and the member of the nitrosourea group of drugs, its main action is selectively exerted upon the β-cells of the pancreas following administration.

Therefore, for the purpose of present study, rats made diabetic by single intraperitoneal injection of streptozotocin (50mg/kg body weight) at day 1 and sacrificed at day 22 were taken as streptozotocin (STZ) control, so that effects of Nigella given orally for 21 days can be assessed and compared.

The differences in serum glucose concentration in both crude Nigella and freeze dried ethanol extract of Nigella treated normal rats when compared to vehicle control were not found statistically significant (P>0.05). However, the serum glucose concentrations in both crude Nigella and freeze dried ethanol extract of Nigella treated STZ-induced diabetic rats were significantly reduced (P<0.001) when compared to those of STZ control diabetic rats.

The results are in agreement with and confirm the earlier observations of hypoglycaemic effects of *Nigella sativa* oil and plant mixture extract containing *Nigella sativa* by other researchers. The mechanism of hypoglycaemic effects of *Nigella sativa* is not known for certain. Fararh et al, demonstrated insulinotropic properties of *Nigella sativa* oil in streptozotocin induced diabetic hamster and concluded that the hypoglycaemic effect of *Nigella sativa* oil resulted at least partly from a stimulatory effect on β-cell function with consequent increase in serum insulin level and that the oil may be a useful therapeutic agent

in the treatment of type-2 diabetes[6]. However, the study of El-Dakhakhny et al, indicated that the hypoglycaemic effects of *Nigella sativa* oil may be mediated by extra pancreatic actions rather than by stimulated insulin release[5]. Meral et al, had earlier shown that treatment with *Nigella sativa* decreased the elevated glucose and malondialdehyde concentrations, increased the lowered glutathione and ceruloplasmin concentrations, and prevented lipid-peroxidation-induced liver damage in diabetic rabbit. It was therefore concluded that *Nigella sativa* might be used in diabetic patients to prevent lipid peroxidation, increase anti-oxidant defense system activity and also prevent liver damage[7]. The present study confirmed the antidiabetic effects of both crude Nigella and its freeze dried ethanol extract but did not offer any clue to its possible mechanism (s), which would require further carefully designed studies.

The present work reveals the hypoglycaemic properties of *Nigella sativa* Linn. (kalajira) seeds with the expectation that hypoglycaemic properties, if inherent in it, would make new, possibly better drug with lesser adverse effects to treat diabetes mellitus in future.

**References :**


1. Cotran R, Kumar V, Robbins SL. The endocrine pancreas. In: Robbins Pathologic Basis of Disease, fourth edition. Philadelphia: W. B. Sounders Co., 1989. pp. 992-1005.
2. Krall LP. The wide world of diabetes. World Book of Diabet Pract, 1986; 2: 501-29.
3. Olefsky JM. The insulin receptor: Its role in insulin resistance of obesity and diabetes. Diabetes 1976; 25: 1154-65.
4. Habib MA, Rashid MU, Gafur MA Asadi ZA. Effects of extracts of *Cajanus cajan* L. on blood glucose and lipid level in streptozotocin-diabetic mice. Bangladesh Journal of Physiology and Pharmacology 2001; 17: 21-3.
5. El-Dakhakhny M, Mady N, Lambert N, Ammon HP. The hypoglycemic effect of *Nigella sativa* oil is mediated by extra pancreatic actions. Res Vet Sci 2002; 73: 279-82.
6. Fararh KM, Atoji Y, Shimizu Y Takewaki T. Insulinotropic properties of *Nigella sativa* oil in streptozotocin plus nicotonamide diabetic hamster. J Vet Med 2001; 48: 593-9.
7. Meral I, Yener Z, Kahraman T, Mert N. Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. J Vet Med 2001; 48: 593-9.
8. Al-Awadi F, Fatania H, Shamte U. The effect of plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. Diabet Res 1991; 18: 163-8.
9. Gunnarsson R, Berne C, Hellerstrom C. Cytotoxic effects of streptozotocin and N-Nitrosomethylurea on the pancreatic β-cells with special regard to the role of Nicotinamide Adenine Dinucleotide. Biochem J 1974; 140: 447-94.