

# Nigella Sativa's Effect on Biochemical as well as Anthropometric Parameters in Diabetic Rats on High Fat Diet

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## ABSTRACT

**Objective:** Black cumin (*Nigella sativa* L.) seeds and its crude extract or essential oils have been widely used traditionally for nutritional and medicinal applications in Asian countries. Its effects on diabetics with high fat diet consumption have not been adequately studied. This study was undertaken to study its effects on body weight, abdominal girth, lipid profile and plasma glucose levels in diabetic high fat diet fed rats. **Materials and Methods :** Streptozotocin induced male Wistar rats were fed high fat diet [5130 kcal] for 4 weeks after which they were given vehicle, *Nigella sativa* ethanolic extract (300mg/kg), *Nigella sativa* ethanolic extract (600 mg) or metformin (100 mg). Lipid profile, blood glucose, body weight & abdominal girth were measured. **Results:** *Nigella sativa* ethanolic extract (600 mg) caused significant reduction in blood glucose, total cholesterol, triglycerides, VLDL and non HDL cholesterol comparable to metformin. **Conclusion :** *Nigella sativa* shows anti-hyperglycemic effects and improvement in lipid profile in diabetic high fat diet fed rats which is comparable to metformin. Further studies are required to advocate its use in patients with diabetes and dyslipidemias.

**KEY WORDS:** Nigella sativa, diabetes mellitus, antihyperlipidemic, antihyperglycemic, high fat.

## Introduction

Diabetes has become a major cause of morbidity and mortality and its global prevalence is growing rapidly. According to International Diabetes Federation there were 366 million people with diabetes in 2011 and this is expected to rise to 552 million by 2030<sup>[1]</sup>. The epidemic of diabetes has been linked to change in modern lifestyle and rise in obesity<sup>[2,3]</sup>. Susceptible over nourished individuals develop type 2 diabetes owing to the failure of adaptive responses

to safely dispose of the fuel surfeit<sup>[4]</sup>. India is the diabetes capital of the world; every fifth diabetic in the world is an Indian<sup>[5]</sup>. Since India has a rich wealth of medicinal plants and the potential to accept the challenge to meet the global demand for them<sup>[6]</sup>, Indian medicinal herbs need to be explored for their efficacy in obese diabetics.

*Nigella sativa* (NS) (Black cumin), an annual herbaceous plant of the Ranunculaceae family, is a commonly used spice in Indian food and has been used for medicinal purposes in Asian and African countries<sup>[7]</sup>. Several beneficial pharmacological properties have been attributed to it such as antihyperglycemic<sup>[8]</sup>, antiobesity<sup>[9]</sup>, antihyperlipidemic<sup>[10]</sup>, antimicrobial<sup>[11]</sup>, antiviral<sup>[12]</sup>, hepatoprotective<sup>[13]</sup> and nephroprotective<sup>[14]</sup> activities. *Nigella sativa* extracts have shown promising results as an antidiabetic agent in Streptozotocin-induced diabetic

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rats by exerting a therapeutic and protective effect on the pancreas, by decreasing oxidative stress and preserving B-cell integrity<sup>[15]</sup>. It has also been found to increase serum insulin, and decrease the diabetes induced hyper-glycaemia<sup>[16]</sup>. It has also shown favorable efficiency on total cholesterol and Low Density Lipoprotein (LDL) reduction and has anti-atherogenic properties<sup>[17]</sup> by two main mechanisms; first uptake of LDL-cholesterol via up regulation of LDL receptor gene and second, inhibition of cholesterol synthesis via suppressing the HMG-COA reductase gene<sup>[18]</sup>. However, to the best of our knowledge, its effect on diabetic rats on high fat diet has not been studied.

In our present research we have studied the effects of NS seeds on lipid profile and plasma glucose level in diabetic rat models that have been fed with high fat diet. These parameters were also studied in experimental animals treated with metformin which helped us to compare the effectiveness of alternative preparation-NS with that of metformin.

## Materials and Methods

### Materials

*N. sativa* seeds were obtained from NBRI (National Botanical Research Institute), Lucknow and were authenticated by a botanist at the same institute. High fat diet (HFD) was composed of 300 g concentrates, 350 corn, 300 g beef tallow, 50 g vitamins, minerals and fibers. Composition of HFD was 20% crude protein, 35% fat, 40 % CHO (starch 35%, 5% sucrose) 5% vitamins and minerals and fibers. Metabolic energy of this diet was 5130 Kcal /kg, 61% of this energy was from fat<sup>[19]</sup>. Streptozotocin was obtained from Sigma Aldrich, USA. Metformin was procured from Novartis, India

### Extract preparation

Ethanol extract of *N. sativa* seeds was prepared by maceration. 50 mg seeds were soaked in 150 ml analytical grade ethanol for 7 days with frequent stirring with a sterile glass rod. After 7 days, extract was filtered using Whatman Filter paper- No. 1 and the filtrate concentrated using Rotary Evaporator and stored at 4°C till further use<sup>[20]</sup>.

### Experimental animals

All experimental procedures were performed after approval from Institutional Animal Ethics Committee and as per the guidelines of Animal Care by, Committee for the Purpose of Control and Supervision

of Experiments on Animals (CPCSEA), India. Male Wistar rats (200-250 gm) were obtained from Central Drug Research Institute, Lucknow. Animals were kept in the institutional animal house under 12 hour day and night cycle under standard conditions. Rats were given food and water ad libitum.

### Experimental design

Diabetes mellitus was induced using Streptozotocin<sup>[21]</sup>. After overnight fasting, single intra-peritoneal injection of Streptozotocin 50mg/Kg was given. Then the animals were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. After a week's time for development of diabetes, diabetic rats (blood glucose above 250 mg/dl) were fed a HFD for 4 weeks and then divided into following four experimental group of 6 rats each.

**Control Group:** Diabetic control was given Distilled Water orally.

***N. sativa* 300:** animals were given *N. sativa* ethanolic extract 300mg/kg body weight orally for 7 days.

***N. sativa* 600:** *N. sativa* ethanolic extract 600mg/kg body weight orally for 7 days.

**Metformin Group:** Metformin 100mg/kg/day orally for 7 days.

### Hypoglycemic and hypolipidemic activity

On Day 0 and Day 7 of the study 1ml of blood was withdrawn from tail vein and sent for analysis of lipid profile and blood glucose. Lipid profile and blood glucose were measured using autoanalyser.

Abdominal circumference (AC)<sup>[22]</sup> was assessed on the largest zone of the rat abdomen using a plastic non extensible measuring tape with an accuracy of 0.1 cm. AC corresponds to visceral fat mass in rodents.

### Statistical analysis

ANOVA was used for comparison of lipid profile, blood glucose, body weight and AC among different groups. Relationships between body weight, AC and response to drug treatment were measured using Pearson's Test.

All the analyses were done using SPSS 17.0 version and P value < 0.05 was considered as significant.

## Results

### Change in body weight and abdominal circumference

There was a significant increase in body weight (BW) and AC of all the DM rats fed on HFD as shown in Table 1. According to table 1.2 no significant difference was seen between the various groups in BW, AC, blood glucose, lipid profile – total cholesterol (TC), High Density Lipoprotein (HDL), Triglyceride (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) – at day 0 before starting treatment. After 7 days of treatment with vehicle, NS 300mg/kg, NS 600 mg/kg or metformin 100mg/kg, no significant difference was seen in BW ( $p > 0.05$ ) or AC ( $p > 0.05$ ).

### Hypoglycemic activity

A highly significant difference ( $p < 0.001$ ) was seen in glucose levels of the various groups.

A significant decrease in blood glucose was seen in rats treated with NS or metformin as compared to control group as is seen in Table 2. No significant difference was seen between the groups taking NS or metformin indicating comparable efficacy of these drugs.

### Effect on lipid profile

#### Total Cholesterol

Highly significant difference ( $p < 0.001$ ) was seen at day 7 in serum cholesterol levels of different groups. Cholesterol levels were significantly lower in groups receiving NS or metformin as indicated by Table 3. No significant difference was seen between NS and metformin groups.

#### High Density Lipoproteins

At day 7, the HDL levels in all the groups were similar ( $p > 0.05$ ) showing that NS or metformin did not significantly affect HDL levels as seen in Table 3.

#### Triglycerides

A highly significant difference was seen between the different groups in TG levels on day 7. However, TG level was significantly lower than control only in group receiving 600 mg/kg NS extract as seen in Table 3.

#### Low Density Lipoprotein

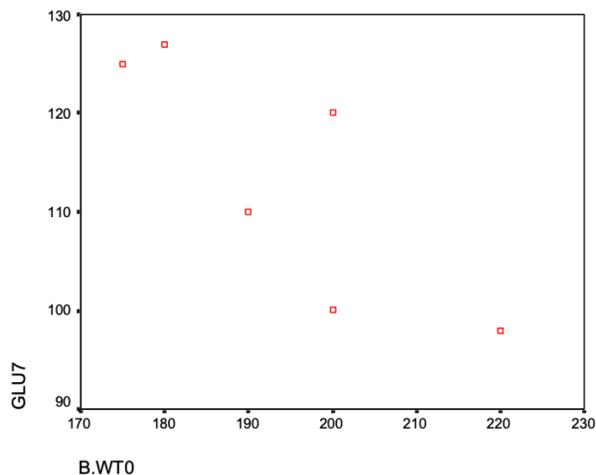
shows highly significant difference ( $p < 0.001$ ) at day 7 in LDL levels of different groups. LDL levels were significantly lower in groups receiving NS or metformin. No significant difference was seen between NS and metformin groups.

#### Very Low Density Lipoprotein

A highly significant difference was seen between the different groups in VLDL levels on day 7 as shown in Table 3. However, VLDL was significantly lower than control only in group receiving 600 mg/kg NS extract.

### Correlation between body weight and effect of *Nigella sativa*

As seen in Table 4 and Figure 1 glucose levels at Day 7 in rats receiving 600 mg/kg NS extract were found to be negatively correlated with their body weight.



**Figure 1: Correlation between BW and B. Glucose in rats administered NS**

## Discussion

Our study is in line with that of Qidwai et al, 2009 [23] who reported that NS seeds reduced BW, BMI and waist-hip ratio, but the results were not statistically significant. In our study a highly significant decrease in glucose levels was seen after administration of NSE which was comparable to metformin. This is in confirmation with the study of Al Awadi FM et al, 1985 [24], who reported a significant decrease in blood

**Table 1: Body weight and abdominal circumference**

Groups	Body weight (g), Mean (CI)		Abdominal Circumference (cm), Mean (CI)	
	Day 0	Day 7	Day 0	Day 7
Control	187.5[168.2-206.8]	196.7[174.0-219.3]	14.3[13.3-12.4]	13.4[12.4-14.4]
NS 300mg/kg	176.7[158.3-195.1]	190.3[168.3-198.4]	13.4[12.7 -14.5]	12.7[12.2-13.2]
NS 600mg/kg	194.2[177.1-211.22]	183.8[183.1-198.6]	12.9[12.1-13.6]	12.6[12.1-13.2]
Metformin	186.7[169.8-203.5]	181.7[167.3-196.0]	13.3[12.1-14.4]	12.7[12.1-13.4]
Anova values				
F	1.073	1.269	0.639	1.788
P	.383	0.312	0.599	0.182

**Table 2: Changes in Blood Glucose and Total Cholesterol**

	Glucose		Total Cholesterol	
	Day 0	Day 7	Day 0	Day 7
Control	331.0 [253.8- 408.1]	374.0 [300.1- 447.8]	83.8n[79.5- 88.1]	89.5 [78.2- 100.8]
NS 300mg/kg	294.8 [242.2- 347.4]	175.8 [123.5- 228.1]	83.5 [78.7- 88.2]	66.4 [61.6- 71.3]
NS 600mg/kg	272.6 [245.9- 299.4]	126.5 [100.1- 113.3]	83.3 [78.8-87.8]	63.5 [60.2- 66.7]
Metformin	297.0 [248.7- 345.2]	128.6 [80.1- 177.3]	84.1 [79.7-88.5]	62.8 [57.4- 68.3]
Anova values				
F	1.303	35.666	0.444	22.347
P	0.301	0.000	0.987	0.000

**Table 3: Changes in Lipid Profile**

	Low Density Lipoprotein [CI]		High Density Lipoprotein		Triglyceride		Very Low-Density Lipoprotein	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Control	6.9 [4.1- 9.8]	6.7 [5.5- 7.9]	49.1 [46.1- 52.2]	50.0 [47.6- 52.3]	138.5 [114-162.3]	166.4 [96.9- 235.8]	27.7 [22.9- 32.4]	12.4 [33.2- 19.4]
NS 300 mg/kg	7.0 [3.6- 10.3]	5.4 [1.7- 9.2]	48.6 [44.5- 52.7]	48.6 [45.8- 50.7]	139.1 [115.2- 163.0]	63.2 [53.8- 72.7]	27.8 [23.0- 32.6]	47.1 [12.6- 10.7]
NS 600 mg/kg	6.1 [2.4- 9.6]	3.7 [0.2- 7.2]	49.6 [47.3- 52.0]	48.3 [45.4- 51.9]	138.0 [115.0- 160.9]	55.5 [48.6- 62.3]	27.6 [23.0- 32.1]	14.5 [11.1- 9.7]
Metformin	7.8 [4.7- 10.8]	3.5 [1.1- 5.9]	48.8 [45.7- 51.9]	45.1 [40.9- 49.3]	137.6 [115.2- 160.1]	71.0 [50.3- 91.7]	27.5 [23.0- 32.0]	14.2 [10.1- 18.3]
Anova values								
F	.315	1.792	.125	2.772	.005	13.251	.005	13.251
P	0.814	0.81	.944	.068	.999	.000	.999	.000

**Table 4: Correlations in NS 600mg.Kg group**

		<b>B.WT0</b>	<b>AC0</b>	<b>GLU7</b>	<b>TC7</b>	<b>LDL7</b>	<b>HDL7</b>	<b>TG7</b>	<b>VLDL7</b>
<b>B.WT0</b>	Pearson Correlation	1.000	.792	-.820	-.429	.227	-.547	-.305	-.305
	Sig. (2-tailed)	.	.060	.046*	.396	.666	.262	.557	.557
	N	6	6	6	6	6	6	6	6
<b>AC0</b>	Pearson Correlation	.792	1.000	-.497	-.467	.081	-.501	-.129	-.129
	Sig. (2-tailed)	.060	.	.316	.351	.878	.312	.808	.808
	N	6	6	6	6	6	6	6	6

glucose produced by a plant mixture containing *N. sativa*.

Our study also confirms the study of Fararh et al., 2002<sup>[25]</sup> and El-Dakhakhny et al., 2002<sup>[26]</sup> who reported anti-hyperglycemic effects of NS oil in Nicotinamide-STZ induced DM hamsters, and STZ DM rats respectively.

In the present study NS effectively reduced TC, TG and VLDL levels. This confirms earlier studies which showed significant decrees in VLDL, TG and TC levels, by NS seeds in Wistar Albino rats<sup>[27]</sup> as well as in humans<sup>[23]</sup>. However, no significant change was seen in HDL and LDL levels. Kocyigit et al, 2009<sup>[18]</sup> reported a significant decrease in LDL and an increase in HDL levels in rats. The difference may be because the rats used in this study were non-diabetic while we used diabetic rats in our study.

A negative correlation was seen between BW before start of treatment and blood glucose levels at the end of NS treatment. This shows that NS is more effective in reducing BG if BW is higher. The reason for this is that higher BW is associated with insulin resistance. NS treatment has been shown to improve glucose tolerance as effectively as metformin<sup>[18]</sup>.

Our study also confirms the study of Najmi A et al<sup>[28]</sup> who reported that NS treatment significantly reduced blood glucose levels in patients of Insulin Resistance Syndrome. The anti-hyperglycemic activity of NS may be due to insulino-tropic<sup>[25]</sup> as well as extra-pancreatic<sup>[26]</sup> effects of NS which may be attributed to the presence of Thymoquinone<sup>[16]</sup>. The antihyperglycemic and hypolipidemic activity of NS coupled with its antioxidant activity against atherogenic diet induced stress<sup>[18,27]</sup> shows that it can be used as an important food supplement in diabetic patients taking high fat diet.

Our study reported superior effect of NSE (600mg/Kg) on Triglyceride levels compared to Metformin, apart

from similar effects on other lipid profile parameters, blood glucose and body weight. Moreover several studies have shown beneficial effects of *Nigella sativa* in prevention or treatment of various comorbidities in people with diabetes, like reduction of CRP levels<sup>[29,30]</sup>, antioxidant activity<sup>[30]</sup>, nephroprotective effect<sup>[31]</sup>, and improvement of endothelial function<sup>[32]</sup>. This indicates the positive implication of using the NSE over metformin

## Conclusion

*Nigella sativa* shows anti-hyperglycemic effects in diabetic high fat diet fed rats which is comparable to metformin. It also causes decrease in TC, TG and VLDL. Although further studies are required to advocate its use in obese DM patients.

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**Conflict of Interest:** None

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## Abbreviations

High density lipoprotein [HDL], *Nigella sativa* ethanolic extract [NSE], triglyceride [TG], very low density lipoprotein [VLDL]

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