# THE EFFECT OF AQUEOUS EXTRACT OF *NIGELLA SATIVA L.* SEEDS IN STREPTOZOCIN INDUCED DIABETIC RAT

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## Summary

The products of the seeds of *Nigella sativa* have been used for many years for therapeutic purposes; several effects such as: anti-inflammatory, anti-microbial, anti-toxic and anti-diabetic have been reported for black seed. Diabetes mellitus is a systemic disease characterized by abnormal metabolic regulation of glucose and lipid, resulting in hyperglycemia and hyperlipidemia. The aim of this study was to investigate the possible effects of *Nigella sativa* seeds on diabetes induced by streptozocin (STZ) in rat.

Forty eight wistar rats were randomly divided into five groups; group 1 received saline and all other groups were received 60mg/kg STZ as ip injection. Three days after STZ injection, group 2 was left untreated while groups 3 and 4 were received 112.5 and 225 mg/kg/day of the aqueous extract in drinking water and group 5 was received 2 mg/kg/day glibenclamid powder orally for 2 weeks. Blood samples from all rats were collected during 4 steps: before STZ injection, three days after STZ injection, one and two weeks after treatment. Serum glucose, cholesterol, triglyceride, urea and creatinine were determined by routine enzymatic biochemical methods spectrophotometrically. Data were expressed as Mean± SEM and were analyzed by one way ANOVA and subsequently by Tukey-krammer; $p<0.05$ was considered significant.

All STZ injected rats were hyperglycemic at 3 days after the injection. By the end of one week treatment, the glucose concentration in groups 4 and 5 decreased significantly ($p<0.001$) *vs* group 2. at the same time the level of triglyceride in group 4, the concentration of urea in groups 4 and 5 and creatinin concentration in groups 3, 4 and 5 were significantly decreased ($p<0.001$, $p<0.01$, $p<0.001$ and $p<0.001$ respectively) when compared with group 2. By the end of the study the differences for all measured parameters in groups 3 and 4 *vs* 2 were not significant.

Our results show that aqueous extract of *Nigella sativa* seeds especially at dose of 225 mg/kg had significant effects on serum glucose, triglyceride, cholesterol, urea and creatinine concentrations; therefore, it may be suggested that *Nigella sativa* seeds may have some beneficial effects in diabetic patients.

**Keywords:** *Nigella sativa,* Diabetes, Blood Glucose, Triglyceride, Cholesterol, Creatinine, Urea.

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## Introduction

*Nigella sativa* L. is an annual plant of the Ranunculacea growing in countries bordering the Mediterranean sea (1). For thousands of years, *Nigella sativa* seeds have been used for culinary and medical purposes; suppression of cough and bronchial asthma and disintegration of renal calculi (2, 3). Several pharmacological properties of *Nigella sativa* seeds including hypotensive, uricosuric, choleretic, anti-oxidant, anti-inflammatory, anti-microbial, anti-toxic, anti-diabetic, and anti-histaminic have been reported (4-8).

Diabetes, is a syndrome characterized by disordered metabolism resulting from either low levels of insulin or from abnormal resistance of tissues to insulin coupled with inadequate levels of insulin secretion to compensate (9, 10)**.** Diabetic patients usually develop sever complications such as: arthrosclerosis, neuropathy and nephropathy with high morbidity and mortality (11). As diabetes affected the level of serum glucose, cholesterol, triglyceride, urea and creatinin, we examined the effects of aqueous extract of *Nigella sativa* seeds on mentioned factors in diabetic rats.

## Methods

***Preparation the aqueous extract of Nigella sativa seeds:***

The *Nigella sativa* seeds collected from Gonabad (Khorasan, Iran) were purchased and recognized by botanist in a herbarium of Ferdowsi University of Mashhad. Seeds were dried and powdered, then 50g of prepared powder was mixed with distilled water and the extract was prepared using suxhelet method (12). The extract was then heated, concentrated and the outcome of the extract relative to *Nigella sativa* powder was calculated as 15%.

***Animal care and treatment:***

All animal procedures were carried out according with the institute of Laboratory Animal Research guide for the care and use of laboratory animals; 48 Wistar rats weighted 200±20g were housed at 22±2°C and 12h light/dark cycle. They were randomly divided into five groups and treated according to the experimental protocol for 2 weeks.

Rats in group 1 (normal control) received saline as ip injection and all other groups received 60mg/kg STZ as ip injection. Three days after STZ injection, group 2 (diabetic control) was left untreated while groups 3 and 4 received 112.5 and 225 mg/kg/day of the aqueous extract in drinking water and group 5 received 2 mg/kg/day glibenclamid powder orally for 2 weeks.

***Blood sampling and analyzing:***

Blood samples from all rats were collected from cavernous sinus during 4 steps: before STZ injection, three days after STZ injection, one and two weeks after treatment. After each step blood samples were centrifuged and the serum was taken and kept in -20 °C. Serum glucose, cholesterol, triglyceride, urea and creatinin were determined by routine enzymatic biochemical methods spectrophotometrically.

***Statistical analysis:***

The data were expressed as Mean± SEM and were analyzed by nonparametric one way ANOVA and Tukey-Krammer test; P-values less than 0.05 were considered significant.

## Results

The serum levels of glucose, triglyceride, cholesterol, urea and creatinin in all rats was measured in 4 steps and then compared together. Results revealed that 3 days after STZ injection, the level of serum glucose in groups 2 (negative control), 3 (treated with 112.5 mg/kg/day of the aqueous extract of *Nigella sativa* seeds), 4 (treated with 225 mg/kg/day of the aqueous extract *Nigella sativa* seeds) and 5 (treated with 2 mg/kg/day glibenclamid) were significantly higher ($p<0.001$) than group 1 (table 1). Meanwhile, the serum level of triglyceride in groups 2 to 5 was significantly higher ($p<0.001$) than group 1 (table 2). Beside hyperglycemia_and hyperlipidemia, rats in groups 2-5 lost weight and show polydipsia and polyuria , which are typical symptoms of diabetic animals.

One week after treatment, the level of serum glucose in groups 4 and 5 significantly decreased ($p<0.001$) compared with group 2 (table 1). Our results showed that 2 weeks after treatment there was no significant difference in the level of serum glucose between groups 2-5.

Table 2 shows that 1 week after treatment, the level of serum triglyceride significantly decreased ($p<0.01$) in group 4 compared with group 2. As it is shown, two weeks after treatment, there was no significant difference between the level of triglyceride in groups 2, 3 and 4, but the level of serum triglyceride was significantly increased ($p<0.001$) in group 5 compared with group 2.

Table 3 shows that one and two weeks after treatment there was no significant difference in the level of serum cholesterol between groups 2, 3 and 4. Whereas, the level of serum cholesterol significantly increased ($p<0.001$) in group 5 compared with group 2 at the same times.

One and two weeks after treatment, the level of serum urea in group 2 was significantly higher ($p<0.001$) than group 1 (table 4). Furthermore, one week after treatment, the level of serum urea in groups 4 ($p<0.01$) and 5($p<0.001$) significantly decreased in compared with group 2; two weeks after treatment, the level of serum urea in groups 4 and 5 was still less than group 2 ($p<0.001$).

Table 5 shows that 3 days after STZ injection, there was no significant difference in the level of serum creatinin between all groups. One week after treatment, the level of serum creatinin in group 2 was significantly ($p<0.001$) higher than group 1 and, at the same time, the level of serum creatinin in groups 3, 4 and 5 was significantly less than group 2 ($p<0.001$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively). By the end of the study the differences for all measured parameters in groups 3 and 4 *vs* 2 were not significant.

**Table 1: Glucose concentration in all groups of rats (mg/dl) during the experiment.**

| Time / Groups | Before STZ injection | Three days after STZ injection | One week after treatment | Two weeks after treatment |
|---|---|---|---|---|
| 1 | 52.2 ± 3.37 | 75.8±11.04 | 95.4±5.47 | 74.8±3.19 |
| 2 | 59.8 ± 3.7 | 302.7± 17.31* | 277.7±37.67 * | 79.6±6.9 |
| 3 | 55.2 ±1.93 | 215.2±29.8 * | 277.5±21.21 | 75.2±36.56 |
| 4 | 63.2 ± 2.5 | 312.2±17.99 * | 97.7±7.25♦ | 76.7±4.8 |
| 5 | 55.6 ± 1.7 | 258.1±15. 37 * | 52±13.87♦ | 60.33±16.85 |

n=8 in group 1 and n=10 in all other groups.

* ($p<0.001$)Significant difference between groups 2-5 vs 1.

♦($p<0.001$)Significant difference between groups 4 and 5 vs 2.

Note: One week after treatment there is no significant difference between groups 4 and 5 vs 1 ($p>0.05$).

**Table 2: Triglyceride concentration in all groups of rats (mg/dl) during the experiment.**

| Time / Groups | Before STZ injection | Three days after STZ injection | One week after treatment | Two weeks after treatment |
|---|---|---|---|---|
| 1 | 102.8 ± 14.16 | 35.83±6.26 | 43.3±5.31 | 36.4±6.94 |
| 2 | 94.33 ± 7.75 | 353± 98.83 * | 109±33.39 ** | 37.5±8.98 |
| 3 | 99.6 ±10.18 | 239.2±56.8 * | 61.7±7.21 | 19.7±7.05 |
| 4 | 128.25 ± 16.26 | ±77.4 * 414.16 | 34±6.94 ♦ | 29.3±7.3 |
| 5 | 86.66 ± 11.39 | ±61.34 * 414.25 | ±10.94 ♦♦ 257.7 | 276±4.35 ♦♦ |

n=8 in group 1 and n=10 in all other groups.
* (p<0.001)Significant difference between groups 2-5 vs 1.
**(p<0.01)Significant difference between group 2 vs 1.
♦(p<0.01)Significant difference between group 4 vs 2.
♦♦(p<0.001)Significant difference between group 5 vs 2.
Note: One week after treatment, there is no significant difference between group 4 vs 1(p>0.05).

**Table 3: Cholesterol concentration in all groups of rats (mg/dl) during the experiment.**

| Time / Groups | Before STZ injection | Three days after STZ injection | One week after treatment | Two weeks after treatment |
|---|---|---|---|---|
| 1 | 41. 6 ± 4.7 | 53.28±7.4 | 54.71±7.59 | 60.5±4.97 |
| 2 | 35.33 ± 8.41 | 50.4±20.61 | 39.75±6.67 | 42.4±8.75 |
| 3 | 27.4 ±5.88 | 51±8.02 | 38.7±5.97 | 32.2±3.46 |
| 4 | 51.5 ± 6.18 | 59.44±6.61 | 53.83±8.62 | 45.25±10.48 |
| 5 | 40.66 ± 6.7 | 61.42±11.85 | 331±5.45 ♦♦ | 328±6.5 ♦♦ |

n=8 in group 1 and n=10 in all other groups.
♦♦(p<0.001)Significant difference between group 5 vs 2.

**Table 4: Urea concentration in all groups of rats (mg/dl) during the experiment.**

| Time / Groups | Before STZ injection | Three days after STZ injection | One week after treatment | Two weeks after treatment |
|---|---|---|---|---|
| 1 | 37.5 ± 5.14 | 29.66±2.77 | 24.4±1.62 | 38.33±1.4 |
| 2 | 35.33 ± 4.7 | 77.5± 7.1** | 80±12.42 ** | 114±6.47 ** |
| 3 | 26.4 ±0.18 | 38.2±4.02 | 86.11±72 | 111.83±9.11 |
| 4 | 36.12 ± 4.38 | 40.24±7.51 | 52±3.13 ♦ | 56.6±8.35 ♦♦ |
| 5 | 38.66 ± 1.7 | 41.42±5.85 | ±12.27 ♦♦ 25.88 | 3.33±0.27 ♦♦ |

n=8 in group 1 and n=10 in all other groups.
**(p<0.01)Significant difference between group 2 vs 1.
♦(p<0.01)Significant difference between group 4 vs 2.
♦♦(p<0.001)Significant difference between groups 4 and 5 vs 2

**Table 5: Creatinine concentration in all groups of rats (mg/dl) during the experiment.**

| Time / Groups | Before STZ injection | Three days after STZ injection | One week after treatment | Two weeks after treatment |
|---|---|---|---|---|
| 1 | 0.49 ± 0.08 | 0.75±0.16 | 1.12±0.96 | 0.42±0.14 |
| 2 | 0.14 ± 0.04 | 2.94± 0.68 | 6.14±1.11 * | 0.98±0.15 |
| 3 | 0.12 ±0.07 | 1.65±0.62 | 3.24±0.62 □ | 1.02±1.04 |
| 4 | 0.16 ± 0.1 | 4.24±1.09 | 0.16±0.1♦ | 0.23±0.01 |
| 5 | 0.99 ± 0.04 | 0.99±0.65 | 0.14±0.05♦ | 0.14±0.04 |

n=8 in group 1 and n=10 in all other groups.
* ($p<0.001$)Significant difference between groups 2 vs 1.
□ ($p<0.05$)Significant difference between groups 3 vs 2
♦($p<0.001$)Significant difference between groups 4 and 5 vs 2

## Discussion

Patients with diabetes are susceptible to an extensive array of medical complications such as retinopathy, nephropathy, neuropathy and arthrosclerosis (13). The result of our study showed that aqueous extract of *Nigella sativa* seeds especially at dose of 225 mg/kg had significant effects on serum glucose similar to glibenclamid, therefore it may have some effects on insulin producing cells.

It was shown that thymoquinon, a product of *Nigella sativa*, has reduced the level of serum glucose in diabetic rats by decreasing the amount of glucose production via gluconeogenesis (14). It was also shown that extracts of *Nigella sativa* enhances glucose induced insulin release from rat langerhans islets (15) and increases the sensitivity of rat liver cells to insulin (2) which confirm the results of our study.

It was shown that *Nigella sativa* extract can protect pancreatic β-cells against cadmium damage (16) and also significantly decrease the amount of necrosis in MNNG-treated rat hepatocytes (17).

As it is shown in table 2, aqueous extract of *Nigella sativa* seeds at doses of 112.5 and 225 mg/kg significantly decreases serum triglyceride, much effective than glibenclamid, and modify the level of serum triglyceride in diabetic rats efficiently. While 3 days after STZ injection and one and two weeks after treatment there was no significant difference between the level of serum cholesterol in groups 2-4 (table 3).

It was shown that 12 weeks after treating with *Nigella sativa* fixed oil, the level of serum triglyceride and cholesterol decreases 22% and 15%, respectively (18). It was also shown that the petroleum ether extracts of *Nigella sativa* decreased the serum level of triglyceride and LDL and also increased the serum level of HDL in diabetic rats after 4 weeks (1), which is in agreement with our findings.

Aqueous extract of *Nigella sativa* seeds at dose of 225 mg/kg decreased the level of serum urea one and two weeks after treatment (table 4). Moreover, aqueous extract of *Nigella sativa* seeds at doses of 112.5 and 225 mg/kg decreased the level of serum creatinine one week after treatment (table 5). It was previously shown that *Nigella sativa* extract decreased the serum level of urea and creatinine (19, 20), which is in favor of our results. In summery our results show that in comparison with glibenclamid, aqueous extract of *Nigella sativa* seeds especially at dose of 225 mg/kg had significant lowering effects on serum glucose, triglyceride, cholesterol, urea and creatinine concentrations; therefore, it may suggested to be used to modify the metabolic complications of diabetes such as hyperglycemia, hyperlipidemia and hyperuremia.

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