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# ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC EFFECTS OF ETHANOL EXTRACT OF *NIGELLA SATIVA* SEEDS IN STREPTOZOCIN / HIGH FAT DIET INDUCED HYPERGLYCEMIC MICE

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## ABSTRACT

**Objective:** The present study was designed to determine the antihyperglycemic and antihyperlipidemic effect of ethanol extract of *Nigella sativa* seeds, in Streptozocin/high fat diet induced hyperglycemic mice. **Materials and methods:** The normal serum values of glucose were determined in 40 healthy male mice before Streptozocin/high fat diet induction &at two occasions 14, 28 day after induction by Streptozocin/ high fat diet and treatment with ethanol extract of *Nigella sativa* seeds for 28 days, also serum C- peptide, cholesterol, triglyceride, VLDL, LDL, HDL are measured at end of experiment. **Results:** *Nigella sativa* showed decrease in serum glucose, cholesterol, triglyceride, VLDL, LDL, also increase in serum C-peptide and serum HDL level measured at day 28 compared with glyburide group also, there is improvement in MDA and GSH serum levels.

**KEYWORDS:** Nigella sativa, Antihyperglycemic, Antihyperlipidemic, STZ, HFD.

## INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia that caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. In spite of the drugs available in the treatment of diabetes and high blood lipids but still accompanied by undesirable side effects, and so a wide attention and tendency for herbal and poly herbal treatment because of the lack of side effects, and low cost and

accessibility. In the present study, the potent DNA alkylating antibiotic streptozotocin (STZ) was used to induce experimentally hyperglycemia in mice[1] (Wu and Yan, 2015).

STZ is a glucosamine–nitrosourea compound derived from Streptomyces achromogenes that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β-cell carcinoma[2] (Graham *et al*., 2011). The results showed that STZ is effective in inducing hyperglycemia and the mechanism of induction involves destruction of β-cells in islets of Langerhans via DNA methylation and subsequent damage after being accumulated inside pancreatic β-cell due to its analogue characteristic to glucose molecule[3] (Kancherla et al., 2011).

High fat diet is used to induce hyperlipidemia in mice[4] (Li x, 2014). *Nigella sativa*, also usually called as "black cumin" have been engaged as a spice and food preservative for thousands years. *N. sativa* seed and oil constituents have shown medicinal properties[5] (Chan, *et al*., 2015).

*Nigella sativa* belongs to the plant family Ranunculaceae. Analysis of the active ingredients of the ethanol extract of *Nigella sativa* seeds proved the presence of different compounds, including of compounds thymoquinon, fatty acids, proteins, alkaloids (nigellicines and nigelledine), and saponins that were examined through GC-MS. Pharmacological studies have proved that *N. sativa* showed a broad variety of biological effects including.

Neuroprotective[6] (Alinejad *et al*., 2013). Cardioprotective[7] (Shafiq *et al*., 2014 ). Anti-cancer[8] (Hosseini and Ghorbani, 2015). And Bronchodialater[9] (Boskabady *et al*., 2010). Antihypertensive[10] (Dehkordi and Kamkhah, 2008). Antioxidant[11] (Kanter *et al*., 2009).

## MATERIALS AND METHODS

**Chemicals:** All chemicals used in the present study were of analytical grade. STZ was procured form Fluke, England. Glyburide from Actavis,England. Cholesterol powder from BDH, England. The kits for estimation of serum C- peptide, MDA, GSH were purchased from Mybiosource, USA.

### Plant extraction

*Nigella sativa* seeds were collected from local herbal apothecary in Baghdad and were authenticated by Botanic Department, Al- Mustansiriya University, Iraq). Then 500 gram freshly collected seed were shade-dried and washed with tap water then washed with distilled

water then it was been dried in an incubator at 37ºc until water droplets completely evaporated then the dried seed were coarsely powdered in mixer grinder. Powdered dried seeds 500 g. were soaked for 15 hr. in 1.5 liters of 95 % ethanol. Then this suspension was filtered and the residue was again soaked in equal amount of ethanol for 48hrs and again filtered. The two filtrates obtained were evaporated and dried by distillation under reduced pressure at 40 to 50°C in rotary evaporator. Complete dryness was done by vacuum pumping. Dried extract was weighted and stored in freezer.

The extraction mass black color so obtained 42 gram was suspended in distilled water in the required amount at the time of administration of experimental animals[12] (Prashant, *et al*., 2011).

**Animals**

Fourty healthy male albino mice weighing 25-40 gm were used in the present study, they were supplied by animal house of college of medicine. Animals were housed under good conditions at 28 Cº in separated cages and were fed high fat diet except control group & were given water.

The mice were randomly allocated to four groups (each contains ten mice) they were given a single daily dose of the followings at 9:00 a.m. for 28 successive days. Group-1 (control) received distilled water (50µl.) orally every day. Group-2 (drug control) received distilled water (50µl.) orally every day and injected with STZ (150 mg/Kg) intraperitonial with HFD. Group-3 received glyburide (p.o) 0.5 mg per kg with HFD as standard drug for 28 days orally. Group-4 received ethanol extract of *Nigella sativa* (300 mg/kg) per day orally. The doses of *Nigella sativa* had been chosen using many doses in pilot study. At 10:00 a.m. of the first day group 2,3 and 4 the animals were injected by streptozotocin (150 mg/kg i.p.). Streptozotocin induces diabetes within 3-5 days by destroying the beta cells of Langerhans islets in the pancreas[13] (Khalifah *et al*.,2005). Prepare the citrate buffer prior to injection, dissolve the STZ in the 50 mM sodium citrate buffer (pH 4.5) to a final concentration of 4 mg/ml. Because STZ degrades within 15 to 20 min after dissolving in the citrate buffer, the STZ solution should be prepared immediately before use[14] (Rehni *et al*., 2017).

Blood samples were collected from tail vein of the mice of all groups for analysis of fasting blood glucose, while blood samples collected from cardiac puncture for biochemical analysis C- peptide, MDA, GSH at the end of experiment using Eliza, while estimation of lipid profile

using autoanalyzer for comparison between the values of these results. Later on, all the mice were sacrificed under light anesthesia of chloroform to take panceas specimen. the histopathological examination was performed to check the microscopic changes of the panceas tissue using polarized microscope after fixating the section in 10% formalin for 48 hours and staining with hematoxylin & eosin.

**Statistical Nalysis**

All the obtained results were expressed as mean ± SEM. The difference among means had been analyzed by student's test using SPSS version 12, p values ≤ 0.05 were considered to be statistically significant.

**RESULTS**

**Gas Chromatography - Mass Spectroscopy (GC-MS) analysis of *Nigella sativa* seeds**

The results obtained from GC-MS analysis for *Nigella sativa* seeds extract are shown in the tables and figures (1.1).

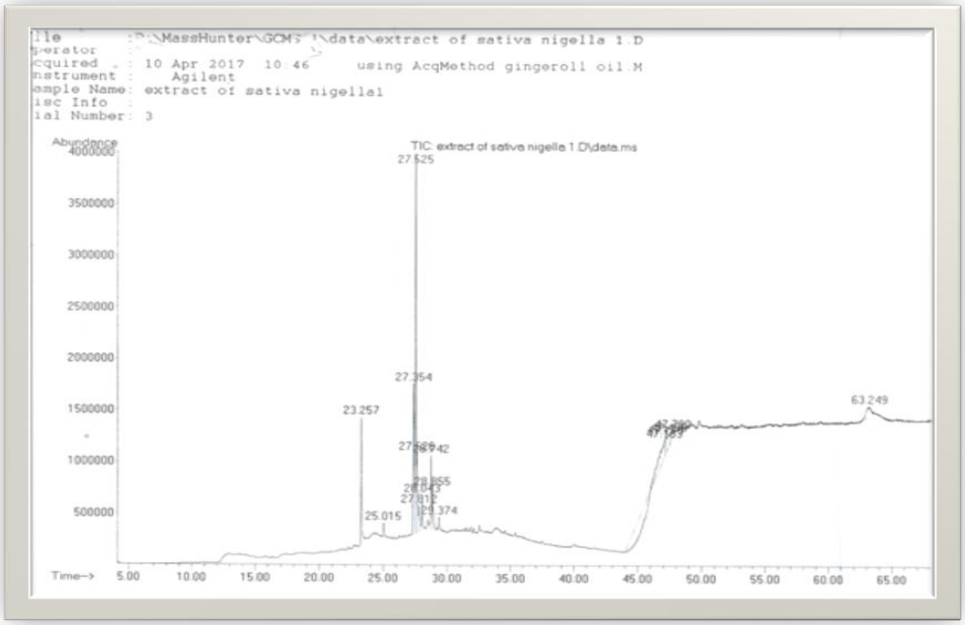


**Figure. (1.1). The chromatogram chart of GC-MS for seeds extract of *N, sativa* seeds.**

**Table. (1-1). The phytochemical constituents detected by GC-MS for *Nigella sativa* with their molecular formula and weight.**

| NO. | Compound name | Molecular formula | Molecular weight |
|---|---|---|---|
| 1 | ß-Pinene | C10H16 | 136 |
| 2 | D-Glucose, 6-O-α- Dgalactopyranosyl | C12H22O11 | 342 |
| 3 | O-Cymene | C10H14 | 134 |
| 4 | DL-Arabinose | C5H10O5 | 150 |
| 5 | Trans -4-methoxy thujane | C11H20O | 168 |
| 6 | 2-Propyltetrahydropyran-3-ol | C8H16O2 | 144 |
| 7 | Terpinen-4-ol | C10H18O | 154 |
| 8 | α- DGlucopyranoside,O-α- D-glucopyranosyl-(1.fwdarw.3)-ß-D-fruc | C18H32O16 | 504 |
| 9 | Thymoquinone | C10H12O2 | 164 |
| 10 | 2-Isopropylidene-5- methylhex-4-enal | C10H16O | 152 |
| 11 | Limonen -6-ol, pivalate | C15H24O2 | 236 |
| 12 | Longifolene | C15H24 | 204 |
| 13 | 2-(4- Nitrobutyryl)cyclooctan one | C12H19NO4 | 241 |
| 14 | ß-Bisabolene | C15H24 | 204 |
| 15 | 1,1-Diphenyl-4- phenylthiobut-3-en-1-ol | C22H20OS | 332 |
| 16 | Phenol, 4-methoxy- 2,3,6-trimethyl | C10H14O2 | 166 |
| 17 | Pyrrolidin -2-one-3ß- (propanoic acid, methyl ester),5- methylene-4α | C16H25NO5 | 311 |
| 18 | l-(+)-Ascorbic acid 2,6- dihexadecanoate | C38H68O8 | 652 |
| 19 | 9,12-Octadecadienoic acid (Z,Z)-, methyl este | C19H34O2 | 294 |
| 20 | 10,13-Eicosadienoic acid, methyl ester | C21H38O2 | 322 |
| 21 | 9-Octadecenamide, (Z) | C15H35NO | 281 |
| 22 | Phthalic acid, decyl oct-3-yl ester | C26H42O4 | 418 |

**Blood glucose concentration and C-peptide level in control and study groups**

Following induction, serial measurements of blood glucose were done on day 14, 21 and 28. In the control group, it was noticed that blood glucose level remained at nearly steady level within normal range, whereas significant changes were reported in study groups as following:

In group (STZ), blood glucose became significantly high on day 14 and continue as such till the end of the experiment with in diabetic range. In all other groups, glyburide, *Nigella sativa*, it was noticed that glucose level started at high level and then became significantly lower on day 14 and further significant reduction was observed on day 28; however the rate of reduction in blood glucose level, when day 28 is taken into consideration, was more marked in the group of glyburide followed by *Nigella sativa*, as shown in table (1-2).

**Table. (1-2): Mean blood glucose in control and study groups at different intervals.**

| Groups | Day 0 | Day 14 | Day 28 |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD |
| Control | 106.25 ±1.51 B, b | 114.75 ±1.99 A, f | 109.50 ±1.82 B, f |
| STZ | 231.50 ±12.06 C, a | 259.50 ± 3.03 A, a | 243.85 ±3.39 B, a |
| Glyburide | 230.01 ±10.88 A, a | 169.50 ± 3.03 B, e | 120.50 ±3.03 C, e |
| Nigella Sativa | 232.50 ±3.03 A, a | 191.00 ±30.28 B, c | 150.50 ±3.03 C, c |

Capital letters for comparison among groups; similar letters for no difference; (A) for the highest value.

At the end of the experiment, C-peptide serum level was estimated for all groups and the results are shown in figure (1-2). Regarding study groups, the lowest concentration was reported in STZ group (0.80 ±0.23) ng/ml and the highest level was recorded in the glyburide group (2.10 ± 0.14) and in *N. sativa* group(2.04 ±0.23) ng/ml.

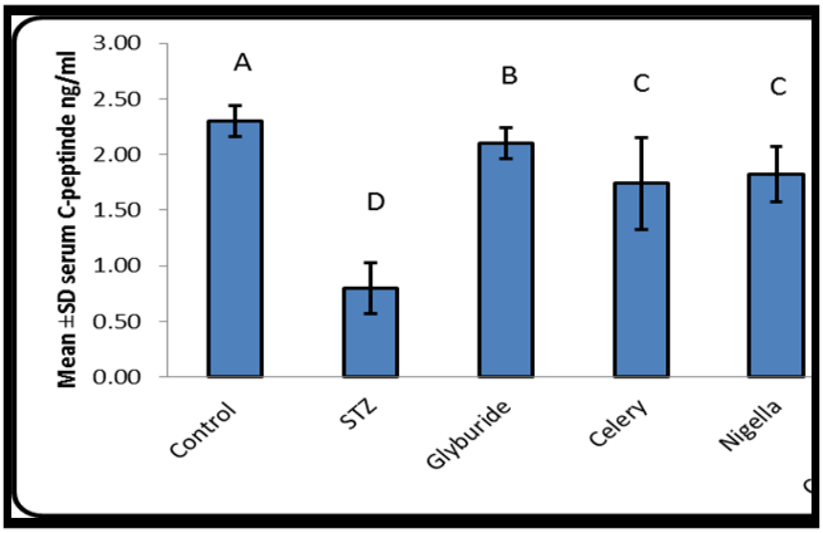


**Figure. (1-2): Serum C-peptide concentration in control and study groups.**

Capital letters for comparison among groups; similar letters for no difference; (A) for the highest value.

The improved serum C-peptide level in groups receiving treatment reflected the improvement in β-cell mass as shown in histological sections (figures 1-3 through 1-6).

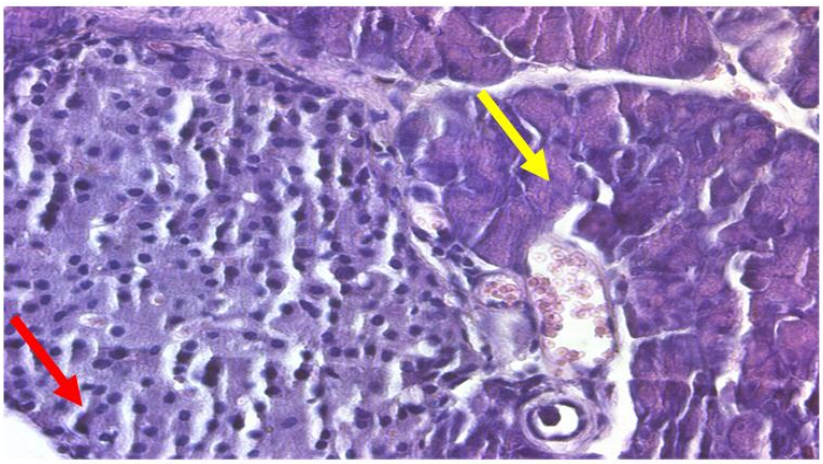

**Figure (1-3): Section of the pancreatic tissue of control group shows normal pancreatic islets (red arrow) and normal pancreatic acini (yellow arrow) 40X.**

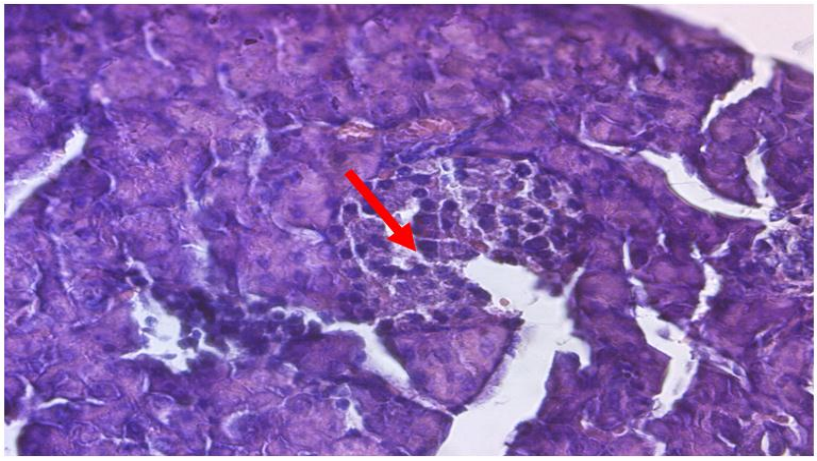

**Figure (1-4): Section of the pancreatic tissue of STZ group shows shrinking of the pancreatic islets and decreases in overall number of pancreatic islets and dilatation of acini (red arrow) 40X.**

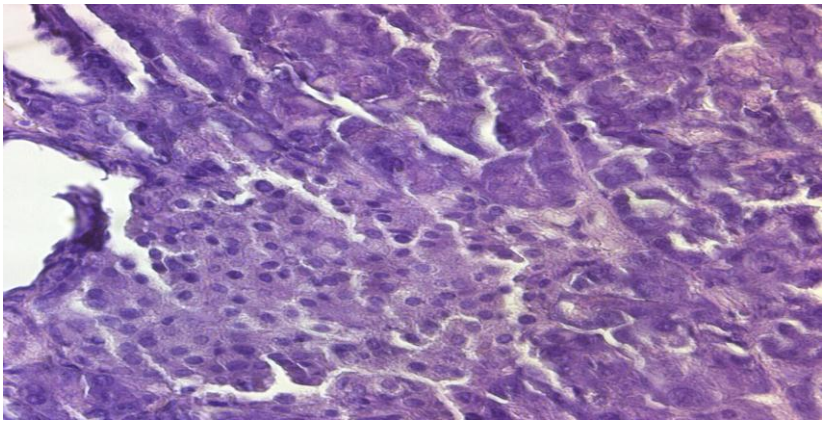

**Figure. (1-5): Pancreatic tissue of the glyburide group section shows improvement in the structure of the pancreatic islets and acini 40X.**

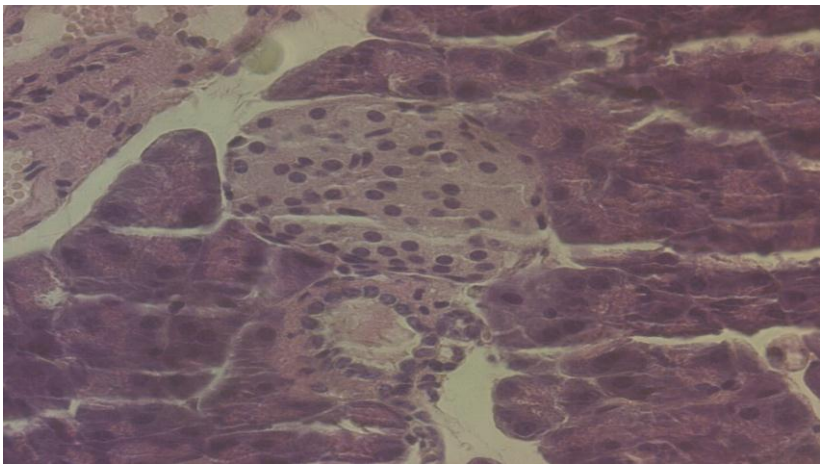

**Figure. (1-6): Section of pancreatic tissue from *N. sativa* group shows improvement in the structure of pancreatic islets and acini, pancreatic islets is a round and oval in shape with smooth outline. There is restoring in normal size and number of the acini also there is recovering to normal morphology 40 X.**

**Fatty liver changes and serum lipid concentration in control and study groups**

Mean serum lipid profile is shown in table (1-3). The highest level of cholesterol, triglyceride, LDL, VLDL, HDL and phospholipid were seen in the group of STZ which was significantly higher than other groups ($P<0.05$). The serum concentrations of cholesterol, triglyceride, LDL,VLDL,HDL and phospholipid in glyburide, *N. sativa* groups were not significantly different ($P>0.05$); however, these levels were significantly higher than that of the control group ($P<0.05$) but it significantly different if compared with STZ group.

**Table. (1-3): Mean serum lipids in control and study groups.**

| Characteristic | Mean ± SD Control group | Mean ± SD STZ group | Mean ± SD Glyburide group | Mean ± SD *N. sativa* seeds group |
|---|---|---|---|---|
| Cholesterol (mg/dl) | 96.40 ±5.30 | 115.67 ±6.70 | 104.70 ±4.30 | 103.15 ±8.70 |
| Triglyceride (mg/dl) | 40.81 ±7.80 | 89.97 ±9.40 | 45.90 ±8.30 | 79.98 ±5.90 |
| LDL (mg/dl) | 32.34 ±8.90 | 37.61 ±7.60 | 34.50 ±5.40 | 33.60 ±5.20 |
| VLDL (mg/dl) | 8.25 ±3.40 | 30.33 ±2.40 | 10.25 ±3.60 | 15.79 ±2.68 |
| HDL (mg/dl) | 59.85 ±5.66 | 18.11 ±7.43 | 53.80 ±6.33 | 44.90 ±3.77 |
| Phospholipid (mg/dl) | 70.13 ±6.77 | 88.99 ±7.34 | 75.50 ±8.35 | 80.60 ±3.03 |

## DISCUSSION

The current study was shown that blood glucose level increased gradually in the group of mice for which STZ was administered orally and the level of blood glucose was reduced following administration of *N. sativa*. For purpose of comparison, glyburide was administered to a group of mice[15] (Moukette *et al.*, 2017).

It was found that mean blood glucose level did not vary significantly in all groups enrolled in the present study at time of diabetes induction. Later on it was found that blood glucose level in the group of mice which received N.S was significantly lower than that of STZ; however it did not reach the level produced by glyburide.

Several authors studied the anti-diabetic effect of N.S in experimentally induced diabetes in animals and found that N.S is efficient in reducing blood glucose level in agreement with result of the present study.

The hypoglycemic effect of *N. sativa* in diabetic animals showed positive results by majority of studies. However, one study reported no significant effect of *N. sativa* on blood glucose[16] (Abdel-Rahman and Abd El-Raouf 1992) the negative results of this study may be due to use low dose of *N. sativa*, which could explain the discrepancy with the current study outcomes.

The proposed mechanism of N.S may be attributable to either intra-pancreatic or extra-pancreatic mechanism.

The intra-pancreatic effect of N.S on Langerhans β-cells may be due to insulinotropic action. In other words, *N. sativa* has the ability to enhance insulin secretion from already existing β-cells. This opinion is supported by the observation of[17] (Mathur *et al*., 2011).

The possible explanation for induced insulin secretion from β-cells is due to the anti-oxidant activity of thymoquinone compound in N.S which protects those cells and induces subcellular changes that favors enhanced insulin secretion Inhibition of intestinal glucose absorption; this effect is mediated by reducing the intestinal sodium-dependent D-glucose cotransporter-1 (SGLT1) which is the major transporter of glucose in the intestine[18] (El-abhar and Shaalan, 2014).

The second possible intra-pancreatic mechanism is through protection of β-cells against oxidative and damage and by this way N.S prevent further damage of the already existing β-cells (Mathur *et al*., 2011).

The third proposed intra-pancreatic mechanism is through induction of pancreatic β-cells regeneration The extra-pancreatic effect in multi-factorial involving insulin like action by enhancing glucose uptake by the liver, muscle and adipose tissue[19] (Benhaddou-Andaloussi *et al*., 2010).

Increase sensitivity to insulin action which is mediated via improving of insulin signaling pathway so that both IGF-1 and PI3K expressions are increased; this affects the signaling molecule Akt that activates GLUT4 and GLUT4 is then translocated to the membrane and imports glucose into the cell[20] (Nagano *et al.*, 2016).

In the present study serum C-peptide level was estimated as an indirect measure for serum insulin concentration since insulin half life is very short as well as exessive first pass effect[21] (Leighton *et al*., 2017). The present study has shown that administration of either or both of N.S causes significant increase in C-peptide level in the serum of mice. This observation is in agreement with[22] (Alimohammadi *et al*., 2016).

The antihyperlipidemic effect of N. sativa seed extract may be attributed to the synergistic effect of its different constituents, soluble fiber, sterols, flavenoids and high content of polyunsaturated fatty acids. The antihyperlipidemic effect of soluble fibers contents of *N. sativa* seeds may be interrelated to decreased cholesterol absorption and increased bile acid synthesis and degradation[23] (Ahmad *et al*., 2015).

Multiple mechanisms of action may in fact contribute to the lipid-lowering effects of *N. sativa*. Earlier, we demonstrated that *N. sativa* was able to regulate cholesterol synthesis through regulation of HMG-CoA reductase and LDL-receptor genes, an effect mediated by TQ and other N. sativa constituents[24] (Al-Naqeep *et al*., 2009).

Other mechanisms of hypolipidemic action of TQ, the active components of N. sativa, have been proposed. TQ was shown to inhibit non-enzymatic lipid peroxidation in liposome and works as a scavenger of various reactive oxygen species including superoxide anion and hydroxyl radicals[25] (Ismail *et al*., 2010).

Antioxidants may also partly contribute to the overall functional effects of N. Sativa particularly, (flavonoids) have been proposed to decrease cholesterol synthesis and suppress reactive oxygen species, nitrogen species formation and protect the antioxidant defense system[26] (Arts and Hollman, 2005). Flavonoids are also thought to enhance the efficiency of liver cells to remove LDL from the blood circulation by increasing LDL receptor densities in the liver and binding to apolipoprotein B[27] (El-Beshbishy *et al*., 2006).

*N. sativa* give a protective effect in diabetes by diminishing oxidative stress and preserving pancreatic beta-cell integrity. *N. sativa* showed its antioxidative effects by the inhibition of

membrane lipid peroxidation and. Some studies have also been conducted on *N. sativa* and, they proposed that this plant increases insulin sensitivity by inhibiting the intensity of oxidative stress. *N. sativa* antioxidant effect appears to be due to its oil, thymoquinone, flavonoids and also antioxidant vitamins like ascorbic acid. It has been shown that the *N. sativa* oil and TQ inhibit non-enzymatic lipid peroxidation in liposomes and both of them especially TQ, work as a scavenger of various reactive oxygen species, including superoxide anion and hydroxyl radicals[28] (Ahmad and Beg**,** 2013).

## CONCLUSION

Ethanol extract of *Nigella sativa* seeds possess mild Antihyperglycemic, Antihyperlipidemic effect in Streptozocin/ high fat diet induced hyperglycemic mice.

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