

Comparative evaluation of *Nigella sativa* (Kalonji) and simvastatin for the treatment of hyperlipidemia and in the induction of hepatotoxicity

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Abstract: Hyperlipidemia is a major risk factor for incidence of coronary artery disease. Simvastatin is a synthetic lipid lowering drug and *Nigella sativa* seeds found helpful in controlling hyperlipidemia. The study performed to evaluate the efficacy of *Nigella sativa* in comparison to simvastatin to treat hyperlipidemia. Thirty Sprague Dawley rats fed on an *ad libitum* diet for 02 weeks, on cholesterol diet for 08 weeks. Then group II treated with simvastatin and group III with *Nigella sativa* for 06 weeks. Blood samples analyzed for serum cholesterol, serum triglycerides, HDL-C, LDL-C & serum ALT. The results evident that *Nigella sativa* (kalonji) and simvastatin showed significant improvement in the lipid profile of rats in respective groups after treatment. The p value <0.05 of group II and III documented that *Nigella sativa* (kalonji) affect the lipid profile in the same way as of simvastatin. However, ALT levels significantly raised in group II treated with simvastatin compared to group III. *Nigella sativa* and simvastatin showed comparable effects in the treatment of hyperlipidemia. *Nigella sativa* showed protective role in terms of hepatic dysfunction and can be used as a cholesterol lowering agent.

Keywords: *Nigella sativa*, Simvastatin, HDL-C, ALT, LDL-C.

INTRODUCTION

Hypercholesterolemia is one of the major risks for heart diseases. The evidence confirmed the association of elevated levels of low-density lipoprotein cholesterol (LDL-C) with increased risk of atherosclerosis (Raal *et al.*, 2011). Therefore, treatment of hyperlipidemia in due course is generally an effective approach to prevent primary and secondary coronary heart disease. Hyperlipidemia is a major contributor to the pathogenesis of cardiovascular diseases. Studies have shown a direct relationship between the incidence of CAD and total and low density lipoprotein cholesterol levels. Hyperlipidemia is a well known cause for the development of CAD without the episode of hypertension, diabetes, smoking, male gender and inflammation (Falk, 2006).

Hyperlipidemia can be controlled through dietary modification or by drug therapies. Several drugs are available to help lower the blood cholesterol. Statins (HMG-CoA reductase inhibitors) effectively lower cholesterol levels to reduce cardiovascular risks (Ma and Han, 2005); (Taylor *et al.*, 2011). All classes of statin considered equally effective regardless of potency and degree to decrease cholesterol (Tonelli *et al.*, 2011). Simvastatin (INN), a member of the statins is a hypolipidemic drug and had shown favorable effects on the pathogenesis of atherosclerosis. It has shown a protective role in preventing cardiovascular morbidity and mortality. Moreover, statins can lower LDL-C concentration which reduces the risk of IHD events and stroke (Law *et al.*, 2003). But some unusual side-effects

such as polyneuropathy, myositis or rhabdomyolysis have been reported as statin negatively effects selenoprotein synthesis (Moosmann and Behl, 2004; Asbach *et al.*, 2009). Similarly, simvastatin effectively controls hypercholesterolemia but showed an adverse effect on liver and muscle (Mukhtar and Reckless, 2005; Armitage *et al.*, 2009; Naci *et al.*, 2013).

Nigella sativa is a medicinal plant traditionally used for bronchial asthma inflammatory diseases, milk production, bronchitis rheumatism, antitumor (Ali and Blunden, 2003; Aggarwal *et al.*, 2008; Banerjee *et al.*, 2009). Physical and chemical analysis showed protein 26.7%, oil 28.48%, ash 4.86% and 40.0% carbohydrate. Linoleic acid and oleic acid are the major polyunsaturated fatty acids with palmitic acid as saturated fatty acid (Khan, 1999; Ramadan and Morsel, 2002; Avula *et al.*, 2010; Michel *et al.*, 2011). The ingredients of seeds of *N. sativa* found effective for reducing tracheal responsiveness and lung inflammation (Boskabady *et al.*, 2011). It also controlled oxidative stress (Ashraf *et al.*, 2011) rheumatoid arthritis (Gheita and Kenawy, 2012) hepatotoxicity (Mansour *et al.*, 2001). *N. sativa* found effective to control hyperlipidemia both in animals and humans (Kocyigit *et al.*, 2009; Attia *et al.*, 2011; Sabzghabae *et al.*, 2012). Earlier data suggested the beneficiary effects of simvastatin and *N. sativa* on lipid profile. However, direct comparison of *N. sativa* with simvastatin without inducing hepatotoxicity is not established for the treatment of hyperlipidemia.

Considering the lipid lowering ability of *N. sativa*, comparison made to evaluate the effectiveness of *N. sativa* with simvastatin. The safety profile of the two

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treatments compared in terms of hepatic dysfunction. The study proposed that *N. sativa* will have comparable effects as of simvastatin without side-effects. Therefore, it can be a better choice for the treatment of hyperlipidemia.

MATERIALS AND METHODS

Randomized comparative trials conducted to compare the efficacy of *N. sativa* with simvastatin for control of hyperlipidemia in rats. A total of thirty (N=30) healthy rats 90–120 days of age included and equally divided into three groups (n=10). Each study group underwent three treatments. Each group administered *ad libitum* diet for one week and blood samples collected after first treatment. These groups shifted on cholesterol rich diet for second treatment to produce hypercholesterolemia in rats. The cholesterol powder added in the diet as 200gm/5kg feed for 08 weeks. The blood samples collected at the end of this treatment. *N. sativa* seeds purchased from a local market. Department of plant sciences, QAU Islamabad did the identification of seeds. In third treatment, group-II administered simvastatin 20mg/kg/day (Tassawar *et al.*, 2011) and group-III administered *Nigella sativa* (1000mg/kg/day). Group I kept as a control on an *ad libitum* diet for 06 weeks. Blood samples collected after six weeks of the treatment. Serum cholesterol, serum triglyceride, serum HDL, serum LDL and serum ALT levels estimated using automated analyzer Selectra. Friedewald formula used for the estimation of serum LDL (Friedewald *et al.*, 1972).

STATISTICAL ANALYSIS

The data statistically analyzed on the spreadsheet of Statistical Package for the Social Sciences (SPSS version 17). The data expressed as mean, standard deviation (SD), standard error. ANOVA followed by Tukey test applied to determine the statistical significant difference for each treatment between three groups. The $p < 0.05$ considered significant.

RESULTS

Body weight of rats

The body weight of the group I on a normal diet noted 213 ± 12.74 gms. The mean weight after the administration of cholesterol diet (second treatment) was 241.5 ± 11.56 gms. The mean weight reduced to 227.5 ± 10.61 gms on *ad libitum* diet in third treatment. The mean value of the weight of group II on an *ad libitum* diet was 215 ± 18.32 g and on cholesterol rich diet 241.5 ± 16.80 g. The 26.5gm average weight increase noted on a cholesterol rich diet after eight weeks. The mean weight after the administration of simvastatin in a normal diet reduced to 215.5 ± 18.32 g respectively after the third treatment. The initial mean weight of rats in group III was 217 ± 11.83 g on *ad libitum* diet, 242.5 ± 14.95 g on a

cholesterol diet and 221.5 ± 12.26 g after the administration of *N. sativa* in normal diet. Therefore, significant increase in weight observed in all three groups when fed on a cholesterol rich diet. However, weight reduction observed in group I when transferred on *ad libitum* diet. Similarly, significant weight reduction noted in-group II & III after the administration of drugs in *ad libitum* diet (fig 1).

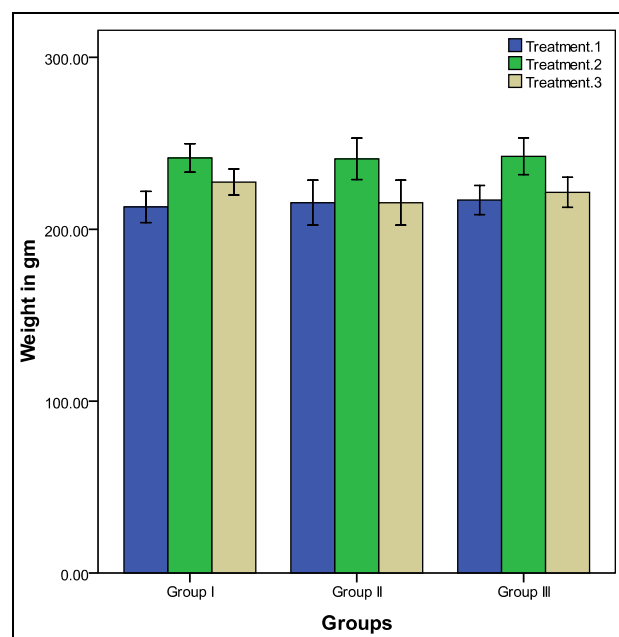


Fig. 1: Body weight difference in three groups of rats. Treatment 1 describes baseline, treatment 2 refers to cholesterol rich diet and treatment 3 refers to drug supplement. Data is expressed as mean $\pm$ SD.

Serum total cholesterol

Group I, II & III did not show remarkable differences in cholesterol levels ($p > 0.05$) after treatment one and two. However, significant differences obtained in cholesterol levels between groups after the third treatments with a p value 0.000 ($p < 0.001$). Significant differences also examined within each group between 1st, 2nd and the 3rd treatment of the study. The mean cholesterol level in group I were 98.4 ± 3.83 mg/dl, 195 ± 5.60 mg/dl and 158.6 ± 10.54 mg/dl after three treatments. Group II showed 100 ± 3.65 mg/dl of cholesterol level after first treatment, 195 ± 6.02 mg/dl after second treatment and 104.6 ± 3.53 mg/dl after third treatment. The mean cholesterol level in-group III was 99.9 ± 3.84 mg/dl after first treatment, 196.5 ± 5.38 mg/dl after second treatment that significantly reduced to 101.4 ± 4.22 mg/dl after *N. sativa* administered (fig 2).

High Density Lipoprotein (HDL)

There was significant difference found in HDL level between three groups. The significant increase in HDL levels noted when *N. sativa* and simvastatin administered to group III & II (p value 0.000) compared to control

group. However, difference was insignificant ($p>0.05$) between groups after first and second treatment. The HDL levels of group I were 39.7 ± 1.42 mg/dl, 20.9 ± 1.45 mg/dl and 31.2 ± 2.62 mg/dl after first, second & third treatment (*ad libitum* diet). The group II HDL levels on *ad libitum* diet noted as 39.9 ± 1.45 mg/dl, reduced to 21.5 ± 1.90 mg/dl after hyperlipidemia. This value again increased to 39.4 ± 1.50 mg/dl after the discontinuation of cholesterol diet and treatment with simvastatin. Statistical significant change detected in HDL levels of rats in this group between three treatments. Mean HDL levels of group III increased to 39.4 ± 1.78 mg/dl from 21.1 ± 1.73 mg/dl after treatment with *N. sativa*. The *ad libitum* level was 39.8 ± 1.55 mg/dl (fig 3). No significant differences observed in HDL levels among group II & III after the administration of simvastatin and *N. sativa* ($p>.05$).

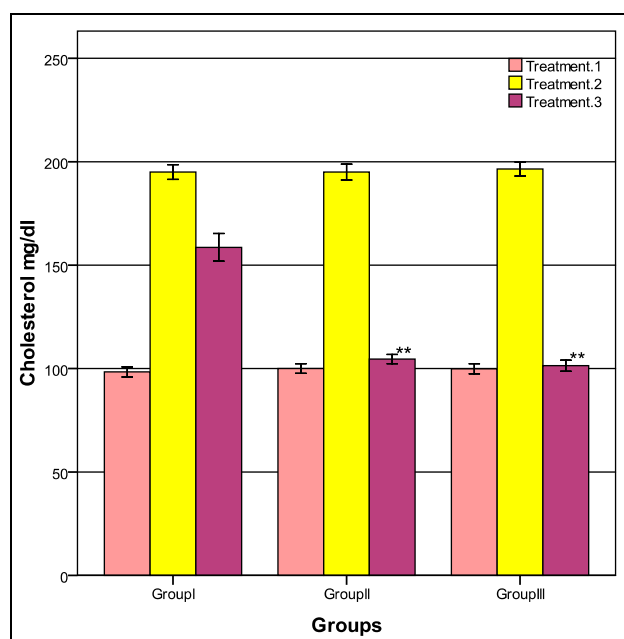


Fig. 2: Cholesterol levels in three groups of rats after three treatments. Data is expressed as mean $\pm$ SD and **represents the $p<0.001$.

Serum Total Triglyceride

A statistical significant difference found in triglyceride levels between three groups after third treatment with p value 0.000. TG levels increased after the induction of hyperlipidemia in all groups. Triglyceride levels dropped to normal range in-group II & III after the administration of simvastatin and *N. sativa* compared to control (group I). Triglyceride levels of group I after first, second and third treatment were 77.6 ± 4.11 mg/dl, 102.2 ± 4.16 mg/dl and 91.5 ± 4.93 mg/dl. The group II triglyceride level on *ad libitum* diet was 77.7 ± 4.24 mg/dl. This level increased to 103.6 ± 4.57 mg/dl after second treatment. TG level significantly reduced to 78 ± 4.12 mg/dl after third treatment. Triglyceride levels of group III after first, second and third treatment were 77.2 ± 4.39 mg/dl, 103.2 ± 3.05 mg/dl and 77.1 ± 4.01 mg/dl. The significant

change in TG levels observed after the treatment with *N. sativa* (fig. 4).

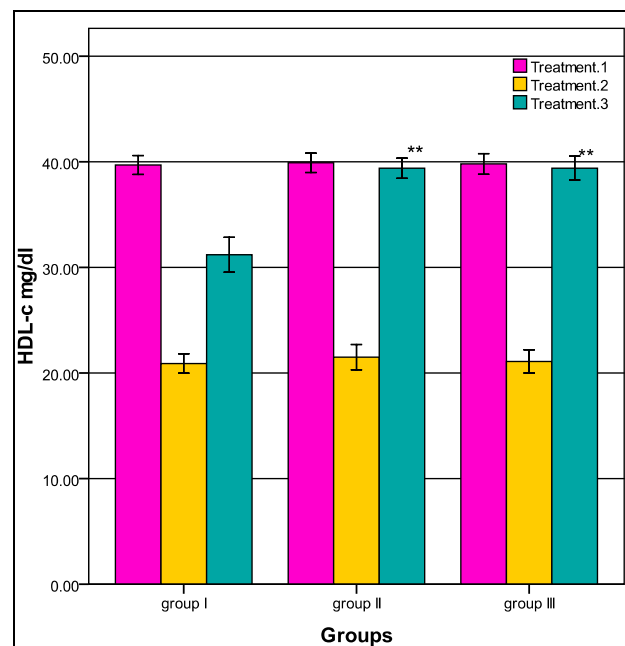


Fig. 3: Measurement of HDL-c levels in three groups of rats after three treatments. Data is expressed as mean $\pm$ SD and **represents the $p<0.001$.

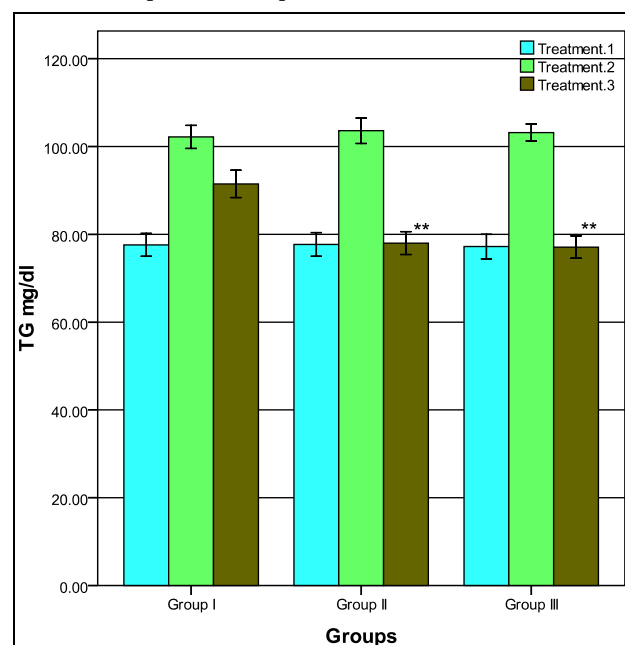


Fig. 4: TG levels in three groups of rats after three treatments. Data is expressed as mean $\pm$ SD and **represents the $p<0.001$.

Low Density Lipoprotein (LDL)

The LDL levels significantly reduced after third treatment with simvastatin and *N. sativa* in group II & III compared to group I ($p<0.001$). The mean LDL levels of group I were 43.18 ± 3.49 mg/dl, 153.66 ± 5.42 mg/dl and $109.1\pm$

11.15mg/dl during the 1st, 2nd and 3rd treatment respectively. Second treatment showed 152.78±6.27mg/dl and reduced to 49.6±4.85mg/dl after third treatment. The LDL level on *ad libitum* diet noted 44.56±3.66mg/dl. Similar results obtained in-group III, the LDL level reduced to 46.58±5.42mg/dl from 154.76±5.55mg/dl after third treatment. The mean LDL level at first treatment was 44.66±3.83mg/dl (fig 5).

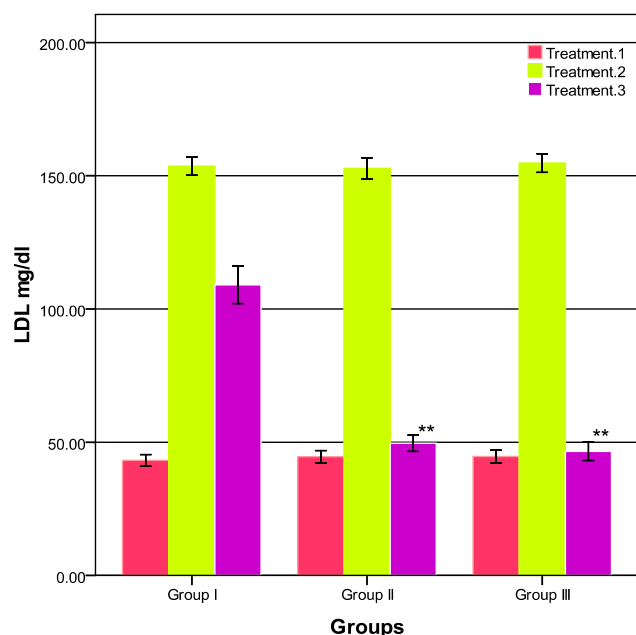


Fig. 5: LDL levels in three groups of rats after three treatments. Data is expressed as mean ± SD and *represents the $p < 0.001$.

Alanine amino transferase

Significant difference noted in ALT levels between three groups ($p < 0.05$) after the third treatment. Group I and III did not show any significant difference (p value > 0.05) after treatment one & three. The mean ALT levels of group I during 1st, 2nd and 3rd treatment were 30.3±7.47 IU/L, 29.7±7.20 IU/L and 29.9±6.23 IU/L respectively. The mean ALT level of group II after the first treatment was 30±8.03 IU/L. The mean ALT values in this group after second treatment was 29.8±6.68 IU/L and increased to 41±12.92 IU/L after the administration of simvastatin (third treatment). ALT levels in group III were 30.2±7.18 IU/L, 29.9±7.58 IU/L and 30.4±5.87 IU/L (fig 6). In this group, ALT levels reached to normal range after the treatment of *N. sativa* while increased above the normal range due to the administration of simvastatin in group II. ANOVA followed by *post hoc* Tukey test illustrated significant differences between group I & II, and I & III ($p < 0.001$) after third treatment. The difference was insignificant between I & II and I & III ($p > 0.05$) after first and second treatment.

DISCUSSION

Present study proved that simvastatin and *N. sativa* maintained the homeostasis of the lipid profile in hyperlipidemic rats. Administration of cholesterol rich diet significantly ($p < 0.01$) increased the weight and affected all parameters of the lipid profile. Results of the present study demonstrated 51% increase in cholesterol and 71.4% in LDL-C levels after feeding on a cholesterol diet. Other studies reported use of coconut oil, soyabean oil, turpentine oil, sheep fat, butter in cholesterol diet to induce hypercholesterolemia (Matos *et al.*, 2005; Rezaq and El-Khamisy, 2011).

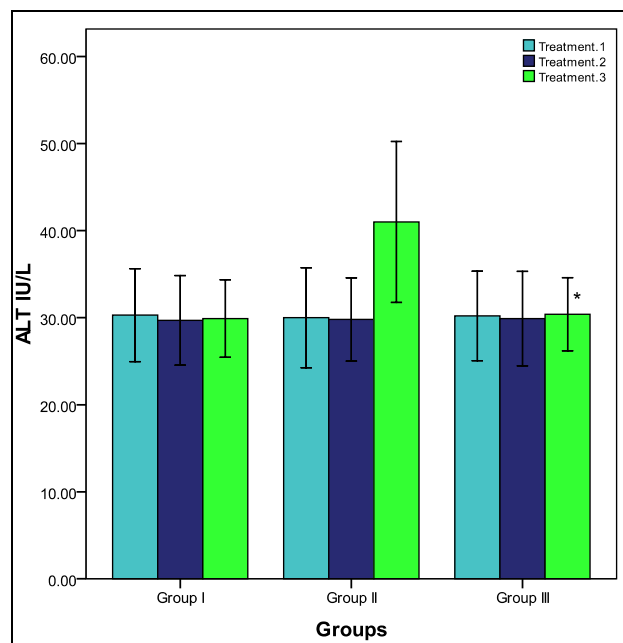


Fig. 6: ALT levels in three groups of rats after treatment 1, 2 & 3. Data is expressed as mean ± SD and * represents the $p < 0.05$.

The current study noted 47% decrease in HDL-C levels after the induction of hyperlipidemia. This difference was due to the supplementation of diet for eight weeks in our study compared to six weeks in other studies. During the third treatment of the present study, simvastatin used in standard dose (20mg/day) as recommended for the treatment of hyperlipidemia. Some studies used high doses up to 80mg/kg but no additional benefits achieved in lowering cholesterol levels and got side effects. The simvastatin produced 46.4%, 24.7% & 67% reduction in serum cholesterol levels, triglyceride and LDL-C levels as compared to control ($p < 0.05$). We also found significant difference in lipid profile within the group before and after the treatment. Moreover, 54% change examined in HDL levels. We obtained significant decrease in TG, LDL, cholesterol levels and significant increase in HDL levels after treatment with simvastatin and *N. sativa*. The duration of hyperlipidemia might have produced significant histological changes (atherosclerosis) in

vessels, which affect the outcome of the treatment (Corti et al., 2005).

The supplementation of *N. sativa* in group III showed a significant change in the lipid profile of the rats. It showed 48.4% reduction in total cholesterol levels. The LDL-C and triglyceride levels reduced to 70% and 25.3% respectively. Moreover, HDL-C levels of rats in this group showed 53.5% improvement after treatment. In accordance with our results, *N. sativa* found an effective lipid-lowering drug in combination with simvastatin (Dahri et al., 2005; Kaatabi et al., 2012). However, we reported here its comparison with simvastatin. The positive impact of *N. sativa* to lower cholesterol, LDL and TG might be due the presence of polyunsaturated fatty acid in seed. The weight reduction after treatment suggested that *N. sativa* has another positive effect to control body weight. We used the seeds in powder form that may contain any other compound effective for weight reduction. Simvastatin is reported to cause some hepatotoxicity. The increased levels of serum ALT after the administration of simvastatin reflected hepatic dysfunction of mild degree. ALT levels remained equal between group I and III (Kanter et al., 2005; Qidwai et al., 2009) after treatment with *N. sativa*. These results confirmed that simvastatin and *N. sativa* are the potential drugs to control the hypercholesterolemia. However, *N. sativa* has protective role in liver function.

In conclusion, *Nigella sativa* and simvastatin has comparable effects in the treatment of hyperlipidemia. Simvastatin is an effective drug to control hyperlipidemia but liver dysfunction could also be precipitated during this treatment. *Nigella sativa* has a better safety profile in terms of hepatic function and would be a better alternate of simvastatin. Large-scale comparative studies on the *Nigella sativa* will be helpful to treat hyperlipidemia without side effects.

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