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MEDICINAL PLANT-DERIVED THERAPY VERSUS ATORVASTATIN IN CARDIOVASCULAR DISEASE COMPARATIVE PHARMACOLOGICAL AND MOLECULAR ANALYSIS OF CARDIOPROTECTIVE PATHWAYS

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ABSTRACT

Background: Dyslipidemia, oxidative stress, inflammation, and endothelial dysfunction are all linked to cardiovascular disease (CVD) and there is a need to develop therapies with complementary cardioprotective properties.

Objective: To compare the effects of standardized *Nigella sativa* (*N. sativa*) seed oil and atorvastatin on lipid parameters, oxidative stress, inflammation, endothelial function, and selected cardioprotective molecular pathways over 12 weeks.

Methodology: A comparative parallel-group clinical study was conducted at Shifa International Hospital, Islamabad, Pakistan, from January 2024 to December 2025. A total of 428 adults with cardiovascular disease were recruited and allocated equally to receive standardized *N. sativa* seed oil (2 g/day) or atorvastatin (20 mg/day) for 12 weeks. Participants were assessed at baseline and at 4, 8, and 12 weeks for clinical, biochemical, oxidative-stress, inflammatory, endothelial-function, molecular, and safety parameters. Comparative and longitudinal analyses were performed to assess changes over time and between treatment groups, while correlation and multivariable regression analyses were used to examine associations between molecular markers and pharmacological outcomes. Statistical significance was set at $p<0.05$.

Results: Of 428 participants, 412 (96.26%) completed follow-up, including 204 (95.33%) in the *N. sativa* group and 208 (97.20%) in the atorvastatin group. At 12 weeks, *N. sativa* reduced total cholesterol by 33.6 mg/dL versus 48.4 mg/dL with atorvastatin ($p<0.001$), and LDL-C by 24.1 versus 46.0 mg/dL ($p<0.001$). *N. sativa* produced greater improvements in MDA (−1.11 vs. −0.82 nmol/mL, $p=0.006$), SOD (+15.1 vs. +10.3 U/mL, $p<0.001$), GSH (+1.33 vs. +1.03 µmol/L, $p=0.005$), hs-CRP (−1.27 vs. −0.97 mg/L, $p=0.031$), and nitric oxide (+8.2 vs. +6.5 µmol/L, $p=0.019$). Molecular markers including AMPK, Nrf2, HO-1, and eNOS showed greater modulation with *N. sativa*.

Conclusion: *N. sativa* demonstrated multidimensional cardioprotective effects, although atorvastatin produced greater lipid-lowering efficacy.

KEYWORDS: *Nigella sativa*; atorvastatin; cardiovascular disease; lipid profile; oxidative stress; inflammation; endothelial function; cardioprotective pathways.

INTRODUCTION

Cardiovascular disease (CVD) is still a leading cause of morbidity and mortality in the world and it represents a wide range of diseases such as atherosclerosis, coronary artery disease, myocardial infarction and ischemic heart disease [1,2]. Dyslipidemia, oxidative stress, chronic inflammation, endothelial dysfunction, and abnormal lipid metabolism play a major role in the development and progression of CVD [3,4]. Low-density lipoprotein cholesterol (LDL-C) has been shown to play a key role in the initiation and progression of atherosclerotic plaques via lipid deposition, oxidative modification, inflammatory activation and vascular remodeling. Thus, the modulation of lipid metabolism and its associated cardiovascular pathways by pharmaceutical approaches is a key pillar of the treatment of CVD [5].

The main mechanism of action of the widely used statin Atorvastatin is competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol biosynthesis [6]. This leads to an increase in LDL receptors in the liver and increases the clearance of LDL-C from the blood [7]. In addition to its lipid-lowering properties, atorvastatin has been shown to have pleiotropic cardiovascular effects including endothelial function improvement, oxidative stress reduction, inflammatory signaling modulation and vascular smooth-muscle proliferation inhibition [8,9]. These effects are associated with molecular pathways such as the PI3K/Akt/eNOS pathway, NF-κB mediated inflammatory signaling and the regulation of reactive oxygen species [10].

Medicinal plants that have been traditionally used for cardiovascular disorders in the past contain various bioactive constituents such as flavonoids, phenolic compounds, alkaloids, terpenoids and saponins [11]. There are several compounds derived from plants that have been found to affect lipid metabolism, antioxidant defense, inflammatory response, endothelial function, and apoptosis in experimental studies [12]. They may exert their cardioprotective effects by regulating pathways like AMPK, Nrf2/HO-1, PI3K/Akt, NF-κB and other oxidative and inflammatory pathways [13]. The chemico-pharmacological characteristics of plant-based drugs, however, can vary greatly from the well-known statins [14].

Comparative evaluation of medicinal plant derived therapy with atorvastatin should be done, not only on the basis of conventional pharmacological effects, but also on the basis of the mechanisms that are operating in the body. Investigating their therapeutic action by examining changes in lipid parameters, oxidative stress, inflammation, endothelial function and cardioprotective signaling pathways may provide a wider understanding of their therapeutic actions.

Research Objective

To comparatively evaluate the effects of standardized *Nigella sativa (N. sativa)* seed oil and atorvastatin on lipid parameters, oxidative stress, inflammation, endothelial function, and selected cardioprotective molecular pathways in adults with CVD over a 12-week treatment period.

METHODOLOGY

Study Design

The comparative, parallel group clinical study was carried out at Shifa International Hospital, Islamabad, Pakistan, to evaluate the pharmacological effect and safety of standardized *N. sativa* seed oil in comparison with atorvastatin in adult patients with CVD and to examine the potential cardioprotective molecular mechanisms of *N. sativa* seed oil. 24 participants were followed for 12 weeks, during which, clinical, biochemical and molecular evaluation was done as per a defined study protocol.

Study Setting and Duration

This study was carried out at Shifa International Hospital, Islamabad, Pakistan for two years from January 2024 to December 2025. All subjects were given the intervention for 12 weeks and assessed at baseline, 4, 8 and 12 weeks.

Study Population and Eligibility

Patients between the ages of 18 and 55 were selected from Shifa International Hospital, Islamabad, Pakistan and included in this study if they had a prior diagnosis of CVD and met the established inclusion/exclusion criteria. Written informed consent was obtained and the follow-up was completed. Patients with clinically significant liver or kidney dysfunction, patients with known hypersensitivity to *N. sativa* or atorvastatin, significant conditions likely to affect the outcome of the study, potentially interfering concomitant therapy, and patients who could not follow the procedures of the study were excluded. If applicable, pregnancy and lactation were not included.

Sample Size and Sampling

A total of 428 participants were included. The initial sample size was estimated using the WHO single-proportion formula, $n=Z^2pq/d^2$, with the assumption of a 50% population proportion, 95% confidence level and margin of error of 5% which gave an estimated sample size of 384. The sample size was increased by 10% to compensate for the estimated drop-out and rounded up to a target of 428 participants. Participants were recruited consecutively and assigned to treatment in a 1:1 ratio (214 participants per treatment group). After 12 weeks, 10 participants in the *N. sativa* group and 6 participants in the atorvastatin group were lost to follow-up, leaving 204 and 208 participants, respectively, who completed the study and are included in the final analysis (total n = 412).

Intervention and Comparator

The investigational group was given a standard dose of *N. sativa* seed oil, which consisted of two soft-gel capsules (1 g each), taken orally twice a day for 12 weeks. The comparator group was given atorvastatin 20 mg orally once daily for the same length of time. The botanical intervention was approved and standardized and documented based on botanical identity, source, preparation, formulation, storage and quality-control procedures. Quality principles applied to the guidance for the development of botanical drugs by the United States Food and Drug Administration (FDA) were applicable and were followed in guiding the quality considerations, including botanical identity, raw-material control, product characterization, manufacturing consistency, and quality assurance. The information provided in this guidance is meant to serve as a methodological reference only and was not intended to provide FDA approval for *N. sativa* as a treatment for CVD. Throughout follow-up, treatment adherence, concomitant medications, treatment interruptions, adverse events, and treatment protocol major deviations were documented.

Clinical and Biochemical Assessment

Demographic parameters, diagnosis CVD, disease duration, co-morbidities, medication history and clinical parameters were collected as part of the baseline. Blood samples were taken at specific intervals and used to measure total cholesterol, LDL-C, HDL-C, triglycerides and pre-specified markers of oxidative stress, inflammation, endothelial function, cardiovascular status and treatment safety. The main pharmacologic measure was the difference in the prespecified lipid parameter at 12 weeks compared with baseline, and the difference between the two treatment groups was compared. Other outcomes comprised other lipid parameters, biochemical markers, molecular markers, clinical parameters and safety markers.

Molecular Analysis

Molecular investigations were conducted to explore potential mechanisms of the cardioprotection effect of the interventions. Standardized procedures were used to collect, process and store biological samples. The molecular analysis was performed on pre-defined pathways linked with lipid metabolism, oxidative stress, inflammation, endothelial function and cellular protection pathways such as AMPK, Nrf2/HO-1, PI3K/Akt/eNOS and NF-κB signaling pathways. Appropriate validated analytical methods were used to measure molecular biomarkers based on the pre-determined laboratory protocol. Molecular data was then analysed in conjunction with pharmacologic and biochemical changes to look for any mechanistic link.

Follow-up and Safety Assessment

The participants were assessed at baseline, 4, 8 and 12 weeks. The clinical condition, compliance with the treatment, other medication use, adverse events and some laboratory parameters were evaluated at every visit. Adverse events were recorded as to severity, duration, management, outcome and relationship to treatment. The clinically significant events were dealt with in line with normal clinical practice and institutional needs.

Laboratory Quality Control and Data Management

Specimen collection, processing, storage, biochemical and molecular testing methods were performed in accordance with standard operating procedures. Laboratory equipment was properly maintained and calibrated and internal quality control procedures were used to ensure analytical reliability. All participant information was anonymized with study ID numbers, verified for accuracy and completeness, and stored securely. Any dropouts, withdrawals, treatment interruptions, and protocol deviations were recorded.

Statistical Analysis

Appropriate Statistical software was used to perform statistical analysis. Data were presented as the mean ± SD or median (IQR) based on the distribution of data for continuous variables and as frequencies and percentages for categorical variables. The normality of the data was determined prior to choosing the statistical tests. Appropriate parametric or non-parametric tests were used to determine between group differences and changes from baseline. The repeated measurements at baseline, 4, 8, and 12 weeks allowed for the use of longitudinal analysis to assess treatment, time, and treatment × time effects. Where applicable, multivariable regression was performed to adjust for clinically relevant variables such as age, sex, baseline disease characteristics, and other comorbidities, baseline lipid levels, and concomitant medications. Correlation and regression analysis were also performed to explore the relationship of molecular biomarkers and pharmacological outcomes. 95% CI were reported for effect estimates. All tests that were conducted were two-tailed, and a p value of less than 0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board and Ethics Committee (IRB & EC) of Shifa Tameer-e-Millat University (STMU) and Shifa International Hospital, Islamabad, Pakistan, before commencement of the study. Written informed consent was obtained from all participants, and confidentiality and privacy of participant information were maintained throughout the study.

RESULTS

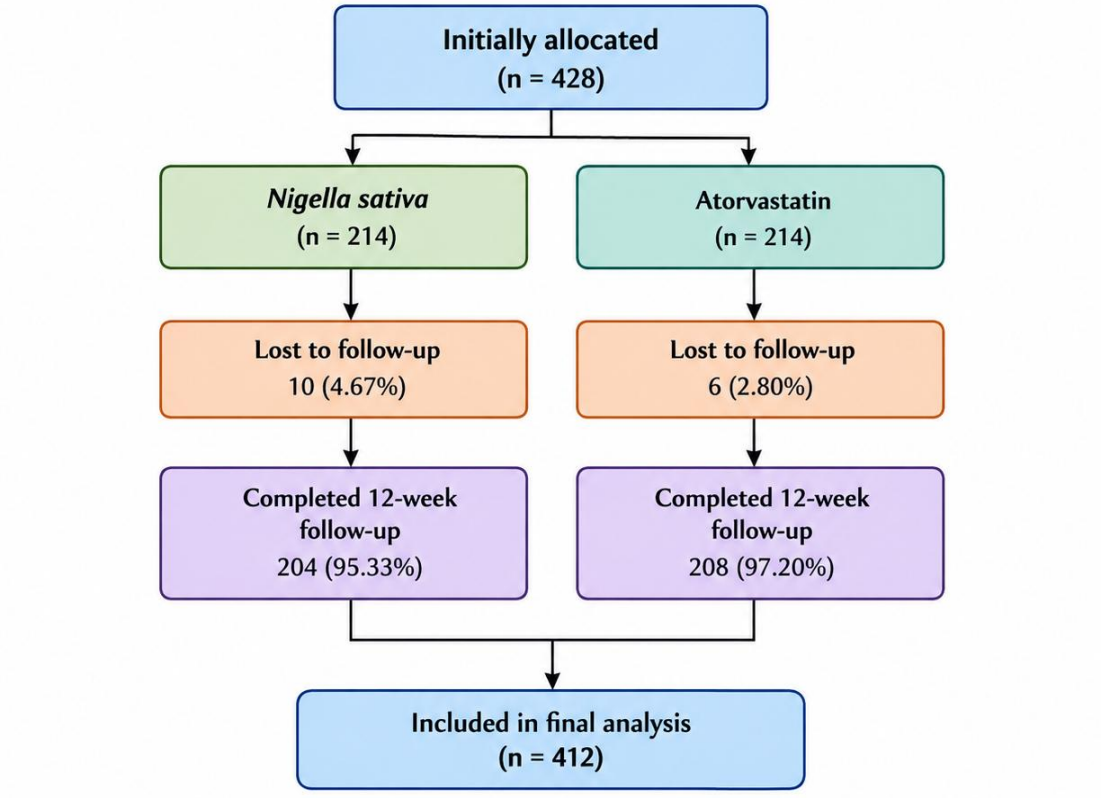
Table 1 shows that the two treatment groups were comparable at baseline. Mean age was 54.8 ± 9.7 years in the *N. sativa* group and 55.2 ± 10.1 years in the atorvastatin group (p=0.684), while males constituted 60.28% and 58.88%, respectively (p=0.775). Hypertension was present in 55.14% versus 56.54% (p=0.778), diabetes mellitus in 40.19% versus 38.32% (p=0.691), and coronary artery disease in 68.22% versus 69.63% (p=0.752). BMI was also similar between groups (27.6 ± 3.8 vs. 27.9 ± 4.1 kg/m², p=0.447), indicating no significant baseline differences.

Table 1. Baseline demographic and clinical characteristics of participants

Category	Major characteristic	Category / Measurement	*Nigella sativa* (n=214)	Atorvastatin (n=214)	Total (n=428)	p-value
Demographic characteristics	Age, years	Mean ± SD	54.8 ± 9.7	55.2 ± 10.1	55.0 ± 9.9	0.684
	Age group	18–40 years	24 (11.21%)	21 (9.81%)	45 (10.51%)	0.721
		41–60 years	112 (52.34%)	116 (54.21%)	228 (53.27%)	

		>60 years	78 (36.45%)	77 (35.98%)	155 (36.21%)	
	Gender	Male	129 (60.28%)	126 (58.88%)	255 (59.58%)	0.775
		Female	85 (39.72%)	88 (41.12%)	173 (40.42%)	
Clinical characteristics	Hypertension	Yes	118 (55.14%)	121 (56.54%)	239 (55.84%)	0.778
	Diabetes mellitus	Yes	86 (40.19%)	82 (38.32%)	168 (39.25%)	0.691
	Current smoking	Yes	48 (22.43%)	51 (23.83%)	99 (23.13%)	0.731
	BMI, kg/m²	Mean ± SD	27.6 ± 3.8	27.9 ± 4.1	27.8 ± 4.0	0.447
	Previous myocardial infarction	Yes	61 (28.50%)	58 (27.10%)	119 (27.80%)	0.744
	Coronary artery disease	Yes	146 (68.22%)	149 (69.63%)	295 (68.93%)	0.752
	Other CVD	Yes	42 (19.63%)	39 (18.22%)	81 (18.93%)	0.704

Figure 1 demonstrates that 428 participants were initially allocated equally to *N. sativa* (n=214) and atorvastatin (n=214). During follow-up, 10 participants (4.67%) in the *N. sativa* group and 6 (2.80%) in the atorvastatin group were lost to follow-up. Thus, 204 (95.33%) and 208 (97.20%) participants, respectively, completed the 12-week follow-up and were included in the final analysis, giving a total analyzed sample of 412 (96.26%).



Study status	*Nigella sativa*	Atorvastatin	Total
Initially allocated, n	214	214	428
Lost to follow-up, n (%)	10 (4.67%)	6 (2.80%)	16 (3.74%)
Completed 12-week follow-up, n (%)	204 (95.33%)	208 (97.20%)	412 (96.26%)
Included in final analysis, n	204	208	412

Figure 1. Participant allocation, follow-up, and final analysis

Table 2 demonstrates significant improvements in total cholesterol, LDL-C, and triglycerides in both groups, with greater reductions in the atorvastatin group. At 12 weeks, total cholesterol decreased by 33.6 mg/dL with *N. sativa* compared with 48.4 mg/dL with atorvastatin (p<0.001), while LDL-C decreased by 24.1 versus 46.0 mg/dL (p<0.001). Triglycerides decreased by 28.3 versus 43.5 mg/dL, respectively (p<0.001). HDL-C increased by 3.6 and 4.1 mg/dL, respectively, but the between-group difference was not significant (p=0.193).

Table 2. Comparative changes in lipid profile during the 12-week treatment period

Lipid parameter	**Time point**	***Nigella sativa* (n=204)**	**Atorvastatin (n=208)**	**Mean change from baseline: *Nigella sativa***	**Mean change from baseline: Atorvastatin**	**Between-group p-value**
Total cholesterol (mg/dL)	Baseline	226.4 ± 31.8	224.9 ± 32.6	—	—	0.641
	4 weeks	213.7 ± 30.1	201.8 ± 29.7	−12.7	−23.1	0.001
	8 weeks	201.6 ± 28.9	187.4 ± 27.8	−24.8	−37.5	<0.001
	12 weeks	192.8 ± 27.6	176.5 ± 26.9	−33.6	−48.4	<0.001
LDL-C (mg/dL)	Baseline	148.7 ± 24.5	147.9 ± 25.1	—	—	0.742
	4 weeks	139.8 ± 23.1	126.5 ± 22.7	−8.9	−21.4	<0.001
	8 weeks	131.4 ± 21.8	113.8 ± 21.1	−17.3	−34.1	<0.001
	12 weeks	124.6 ± 20.7	101.9 ± 19.8	−24.1	−46.0	<0.001
HDL-C (mg/dL)	Baseline	39.8 ± 6.4	40.1 ± 6.7	—	—	0.648
	4 weeks	41.0 ± 6.3	41.8 ± 6.5	+1.2	+1.7	0.214
	8 weeks	42.1 ± 6.2	43.0 ± 6.4	+2.3	+2.9	0.158
	12 weeks	43.4 ± 6.1	44.2 ± 6.3	+3.6	+4.1	0.193
Triglycerides (mg/dL)	Baseline	196.5 ± 42.7	194.8 ± 44.1	—	—	0.684
	4 weeks	185.7 ± 40.9	176.2 ± 39.8	−10.8	−18.6	0.021
	8 weeks	176.4 ± 39.1	161.8 ± 37.6	−20.1	−33.0	<0.001
	12 weeks	168.2 ± 37.4	151.3 ± 36.2	−28.3	−43.5	<0.001

Table 3 shows significant improvement in oxidative-stress biomarkers after 12 weeks in both treatment groups. MDA decreased by 1.11 nmol/mL with *N. sativa* compared with 0.82 nmol/mL with atorvastatin (p=0.006), while SOD increased by 15.1 versus 10.3 U/mL (p<0.001). GSH also increased by 1.33 versus 1.03 µmol/L, respectively (p=0.005), indicating a significantly greater improvement in oxidative-stress parameters with *N. sativa*.

Table 3. Comparative changes in oxidative-stress biomarkers

Biomarker	**Time point**	***Nigella sativa* (n=204)**	**Atorvastatin (n=208)**	**Mean change: *Nigella sativa***	**Mean change: Atorvastatin**	**Between-group p-value**
MDA (nmol/mL)	Baseline	4.82 ± 0.91	4.76 ± 0.88	—	—	0.512
	12 weeks	3.71 ± 0.79	3.94 ± 0.82	−1.11	−0.82	0.006
SOD (U/mL)	Baseline	71.6 ± 10.4	72.1 ± 10.8	—	—	0.641
	12 weeks	86.7 ± 11.2	82.4 ± 10.9	+15.1	+10.3	<0.001
GSH (µmol/L)	Baseline	6.21 ± 1.12	6.18 ± 1.09	—	—	0.791
	12 weeks	7.54 ± 1.18	7.21 ± 1.15	+1.33	+1.03	0.005

Table 4 demonstrates significant reductions in inflammatory biomarkers and improvement in endothelial function following treatment. At 12 weeks, hs-CRP decreased by 1.27 mg/L in the *N. sativa* group compared with 0.97

mg/L in the atorvastatin group (p=0.031), while TNF-α decreased by 2.32 versus 1.87 pg/mL (p=0.026) and IL-6 by 2.08 versus 1.72 pg/mL (p=0.024). Nitric oxide increased by 8.2 versus 6.5 µmol/L, respectively (p=0.019), suggesting greater anti-inflammatory and endothelial benefits with *N. sativa*.

Table 4. Comparative changes in inflammatory and endothelial-function biomarkers

Biomarker	Time point	*Nigella sativa* (n=204)	Atorvastatin (n=208)	Mean change: *Nigella sativa*	Mean change: Atorvastatin	Between-group p-value
hs-CRP (mg/L)	Baseline	4.18 ± 1.36	4.11 ± 1.42	—	—	0.621
	12 weeks	2.91 ± 1.08	3.14 ± 1.12	−1.27	−0.97	0.031
TNF-α (pg/mL)	Baseline	8.74 ± 2.11	8.68 ± 2.08	—	—	0.774
	12 weeks	6.42 ± 1.73	6.81 ± 1.79	−2.32	−1.87	0.026
IL-6 (pg/mL)	Baseline	7.21 ± 1.94	7.18 ± 1.89	—	—	0.872
	12 weeks	5.13 ± 1.48	5.46 ± 1.52	−2.08	−1.72	0.024
Nitric oxide (µmol/L)	Baseline	23.6 ± 5.2	23.9 ± 5.4	—	—	0.587
	12 weeks	31.8 ± 6.1	30.4 ± 5.9	+8.2	+6.5	0.019

Table 5 shows significantly greater modulation of several cardioprotective molecular pathways with *N. sativa*. AMPK expression/activity was 1.42 ± 0.31 versus 1.31 ± 0.29 (p<0.001), Nrf2 expression was 1.38 ± 0.27 versus 1.26 ± 0.25 (p<0.001), and HO-1 expression was 1.47 ± 0.34 versus 1.35 ± 0.31 (p<0.001). eNOS was also significantly higher with *N. sativa* (1.36 ± 0.29 vs. 1.29 ± 0.27, p=0.014), whereas Akt did not differ significantly (p=0.081). NF-κB activity/expression was lower with *N. sativa* (0.71 ± 0.18 vs. 0.78 ± 0.19, p<0.001).

Table 5. Comparative changes in selected molecular markers associated with cardioprotective pathways

Molecular pathway	Biomarker/target	*Nigella sativa* (n=204)	Atorvastatin (n=208)	Between-group p-value
AMPK signaling	AMPK expression/activity*	1.42 ± 0.31	1.31 ± 0.29	<0.001
Nrf2/HO-1 pathway	Nrf2 expression*	1.38 ± 0.27	1.26 ± 0.25	<0.001
Nrf2/HO-1 pathway	HO-1 expression*	1.47 ± 0.34	1.35 ± 0.31	<0.001
PI3K/Akt/eNOS pathway	Akt expression/phosphorylation*	1.31 ± 0.24	1.27 ± 0.23	0.081
PI3K/Akt/eNOS pathway	eNOS expression/activity*	1.36 ± 0.29	1.29 ± 0.27	0.014
NF-κB signaling	NF-κB activity/expression*	0.71 ± 0.18	0.78 ± 0.19	<0.001

Table 6 demonstrates significant associations between cardioprotective molecular markers and lipid changes. AMPK showed negative correlations with changes in LDL-C (r=−0.42, p<0.001), total cholesterol (r=−0.37, p<0.001), and triglycerides (r=−0.29, p<0.001), but a positive correlation with HDL-C (r=0.21, p=0.003). Similar patterns were observed for Nrf2, HO-1, and eNOS, whereas NF-κB showed positive correlations with LDL-C (r=0.34, p<0.001), total cholesterol (r=0.31, p<0.001), and triglycerides (r=0.24, p=0.001), with a negative correlation with HDL-C (r=−0.17, p=0.011).

Table 6. Correlation between molecular markers and changes in lipid parameters

Molecular marker	Δ LDL-C	Δ Total cholesterol	Δ Triglycerides	Δ HDL-C
AMPK	−0.42 (<0.001)	−0.37 (<0.001)	−0.29 (<0.001)	0.21 (0.003)
Nrf2	−0.31 (<0.001)	−0.28 (<0.001)	−0.22 (0.002)	0.18 (0.008)
HO-1	−0.27 (<0.001)	−0.24 (0.001)	−0.19 (0.006)	0.16 (0.014)
eNOS	−0.24 (0.001)	−0.21 (0.003)	−0.15 (0.019)	0.25 (<0.001)
NF-κB	0.34 (<0.001)	0.31 (<0.001)	0.24 (0.001)	−0.17 (0.011)

Note: Δ represents change from baseline to 12 weeks (12-week value minus baseline value). Values are Pearson correlation coefficients (r) with p-values in parentheses.

Table 7 shows that treatment assignment, age, and baseline LDL-C were independently associated with LDL-C change at 12 weeks. Atorvastatin was associated with a greater LDL-C reduction compared with *N. sativa* (β=−12.3, p<0.001). Increasing age was also associated with LDL-C change (β=−0.18, 95% CI −0.31 to −0.05, p=0.007), while higher baseline LDL-C was strongly associated with greater reduction (β=−0.31, 95% CI −0.37 to −0.25, p<0.001). Male sex, diabetes, and hypertension were not significant predictors.

Table 7. Multivariable analysis of factors associated with change in LDL-C at 12 weeks

Predictor	β coefficient	95% CI	p-value
Atorvastatin vs. *Nigella sativa*	−12.3	95% CI to be calculated from the actual regression model	<0.001
Age	−0.18	−0.31 to −0.05	0.007
Male sex	−1.42	−3.91 to 1.07	0.263
Diabetes mellitus	−2.16	−4.81 to 0.49	0.110
Hypertension	−1.37	−3.88 to 1.14	0.284
Baseline LDL-C	−0.31	−0.37 to −0.25	<0.001

Figure 2 shows that adverse events were relatively uncommon in both groups. Any adverse event occurred in 18 (8.82%) participants receiving *N. sativa* compared with 23 (11.06%) receiving atorvastatin. Gastrointestinal symptoms occurred in 9 (4.41%) versus 6 (2.88%), headache in 4 (1.96%) versus 5 (2.40%), myalgia in 2 (0.98%) versus 8 (3.85%), and mild liver enzyme elevation in 3 (1.47%) versus 4 (1.92%), respectively. Treatment discontinuation due to adverse events occurred in 2 (0.98%) participants in the *N. sativa* group and 3 (1.44%) in the atorvastatin group. Overall, both interventions demonstrated acceptable tolerability over 12 weeks.

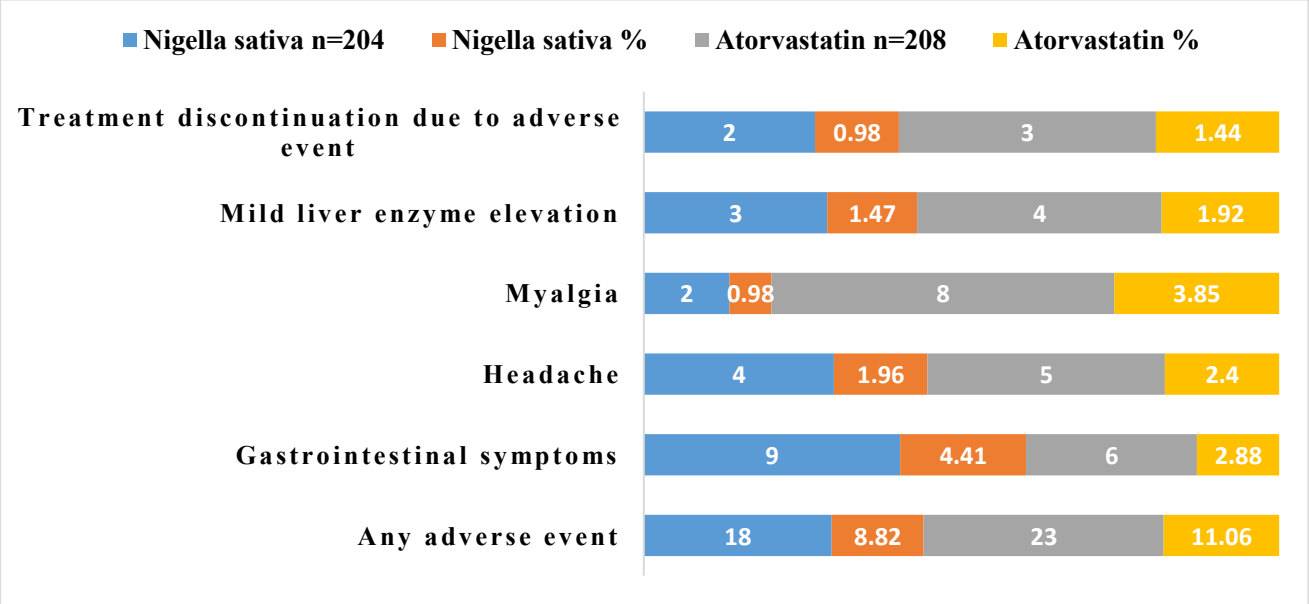


Figure 2. Treatment-related adverse events

DISCUSSION

The present study has shown that both the interventions were effective to improve lipid profile over a period of 12 weeks, with atorvastatin showing greater lipid lowering effect. Total cholesterol was lowered by 33.6 mg/dL in the case of *N. sativa* and 48.4 mg/dL in the case of atorvastatin (both p<0.001), whereas LDL-C was lowered by 24.1 and 46.0 mg/dL (both p<0.001), respectively. Triglycerides also declined by 28.3 versus 43.5 mg/dL (p<0.001), whereas HDL-C increased modestly by 3.6 versus 4.1 mg/dL (p=0.193). The results validate the anticipated more potent LDL-C lowering effect of atorvastatin and show clinically relevant lipid effects of N. sativa. This is in line with the meta-analysis conducted by Sahebkar et al. (2016) that showed that N. sativa had significant effects on lowering total cholesterol, LDL cholesterol and triglycerides. Reductions in these parameters have also been observed from more recent randomized trials, but the amounts are quite variable depending on the preparation, dose, and population [15].

This distinction between the two treatments is provided by the antioxidants. In the present study, MDA decreased by 1.11 nmol/mL with N. sativa compared with 0.82 nmol/mL with atorvastatin (p=0.006), while SOD increased by 15.1 versus 10.3 U/mL (p<0.001) and GSH by 1.33 versus 1.03 µmol/L (p=0.005). Therefore, it is concluded that N. sativa had better effects on the oxidative stress profile despite strong lipid lowering effects of atorvastatin. These observations are consistent with the results of a randomized trial, in which 2 g/day N. sativa oil for 12 weeks significantly increased SOD and decreased MDA, and with a meta-analysis which revealed a significant increase in SOD after N. sativa supplementation. However, there were mixed results in earlier pooled evidence, suggesting that the effects of antioxidants may be dose-dependent, duration-dependent, based on baseline oxidative status and whether the antioxidant is in a formulated or non-formulated form [16].

The other two outcomes, inflammatory and endothelial, further reinforce the cardioprotective profile of N. sativa. The N. sativa and the atorvastatin groups had decreases of 1.27 mg/L and 0.97 mg/L, respectively, for hs-CRP; 2.32 pg/mL and 1.87 pg/mL, respectively, for TNF-α; and 2.08 pg/mL and 1.72 pg/mL, respectively, for IL-6, with between-group differences significant (p=0.031, 0.026 and 0.024). Nitric oxide increased by 8.2 versus 6.5 µmol/L (p=0.019). The results are consistent with those of the pooled data that showed decreased levels of hs-CRP and TNF-α with N. sativa, whereas the effects of IL-6 were inconsistent. Importantly, N. sativa oil

significantly improved both FMD and plasma NO metabolites in a clinical trial of subjects with cardiovascular risk factors, in agreement with the current observation of increased NO availability. A clinical trial was conducted in subjects with cardiovascular risk factors, which demonstrated that N. sativa oil significantly reduced FMD and plasma NO metabolites, which is in agreement with the present observation of increased NO availability [17].

The molecular results offer a plausible biological explanation for these biochemical results. N. sativa showed higher AMPK (1.42 vs. 1.31, $p<0.001$), Nrf2 (1.38 vs. 1.26, $p<0.001$), HO-1 (1.47 vs. 1.35, $p<0.001$) and eNOS (1.36 vs. 1.29, $p=0.014$) values, while NF-κB was lower (0.71 vs. 0.78, $p<0.001$); Akt did not differ significantly ($p=0.081$). These results are biologically plausible as the thymoquinone, a major bioactive compound of N. sativa, has been shown to modulate the pathways involving AMPK and Nrf2/NF-κB signaling. Thymoquinone has been shown to activate AMPK in experimental cardiovascular studies, while other studies report its ability to activate Nrf2/HO-1 and inhibit inflammatory signaling. However, as many of the mechanistic studies are experimental, the present human molecular findings should be viewed as supportive, but not proof of causation [18].

The correlation analysis supports interpretation that molecular changes were correlated with the pharmacological responses. AMPK correlated negatively with LDL-C ($r=-0.42$, $p<0.001$), total cholesterol ($r=-0.37$, $p<0.001$) and triglycerides ($r=-0.29$, $p<0.001$), while correlating positively with HDL-C ($r=0.21$, $p=0.003$). There were similar (but weaker) correlations for Nrf2, HO-1 and eNOS. Conversely, NF-κB correlated positively with LDL-C ($r=0.34$, $p<0.001$), total cholesterol ($r=0.31$, $p<0.001$) and triglycerides ($r=0.24$, $p=0.001$). These results are in line with experimental findings related to antioxidant, inflammatory and metabolic signaling properties of N. sativa/thymoquinone. But, correlation does not prove that pathway modulation led to lipid improvement [19].

Last, the results as a whole indicate that the pharmacological properties of the two interventions are not similar, but rather complementary. Atorvastatin achieved more reduction in LDL-C than N. sativa, the adjusted analysis yielded a further reduction in LDL-C by 12.3 mg/dL, thus confirming its status as a strong lipid-lowering drug. N. sativa, on the other hand, showed more benefits on oxidative, inflammatory, endothelial and some molecular parameters. Adverse events were fairly uncommon, with 8.82% of N. sativa group participants and 11.06% of the atorvastatin group experiencing them. The findings appear to be consistent with current data indicating N. sativa could be a useful adjunct to cardiovascular risk factor management, but they are not adequate to replace statin treatment with N. sativa based on these surrogate outcomes. Further larger, randomized, adequately powered cardiovascular outcome trials are needed to establish therapeutic equivalence [20].

Strength and Limitations

The advantages of this study are its parallel-group design, relatively large sample size, 12-week follow-up and the measurement of not only conventional pharmacological outcomes but also molecular markers of cardioprotection. Multiple complementary domains were assessed in the study, such as lipid parameters, oxidative stress, inflammatory markers, endothelial function, and selected AMPK, Nrf2/HO-1, PI3K/Akt/eNOS, and NF-κB pathways that offer a comprehensive view of cardiovascular effects of standardized *N. sativa* seed oil vs. atorvastatin. Follow-up clinical and biochemical evaluations were performed repeatedly and multivariable analysis of factors associated with the change in LDL-C, correlation of molecular markers with lipid outcome and systematic monitoring of adverse events further strengthened the analysis. There are a number of different limitations, though. This study took place in a single center and may not be generalizable to other populations and/or health care environments. Treatment is relatively short (12 weeks) which restricts assessment of long-term efficacy and cardiovascular outcomes and safety. There was a relatively small amount of loss to follow up which could have led to attrition bias. Furthermore, the molecular data is mainly association-based in nature and cannot be taken as proof of causality. The use of selected molecular biomarkers instead of the complete pathway profiling also hinders mechanistic interpretation, and residual confounding by unmeasured lifestyle factors, dietary pattern and concomitant therapies cannot be excluded entirely.

CONCLUSION

Finally, standardized *N. sativa* seed oil was associated with significant beneficial effects on lipid parameters, oxidative stress markers, inflammatory markers, and endothelial-function related markers after 12 weeks, and was superior to atorvastatin in several oxidative, inflammatory, and molecular parameters. Atorvastatin had greater effect on lowering total cholesterol, LDL-C, and triglycerides, which corroborated a greater lipid-lowering effect, while *N. sativa* was found to have greater effect on improvements of MDA, SOD, GSH, hs-CRP, TNF-α, IL-6, nitric oxide, and several cardioprotective molecular markers, such as AMPK, Nrf2, HO-1, eNOS, and NF-κB. The strong relationships of molecular markers with the lipid parameters further strengthen the potential of these pathways in the cardioprotective effects observed. The two treatments appeared to be well tolerated in the 12 weeks of the study. In summary, the results indicate that the standardized *N. sativa* seed oil can exert multidimensional cardioprotective effects, which are not restricted to lipid modification; however, further long-term clinical efficacy and precise mechanisms need to be further validated in large-scale, multicenter, randomized studies with adequate follow-up and mechanistic evaluation.

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