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Assessing the Impact of Processed *Nigella sativa* and Chia Seed Powder on Nutrient Bioavailability and Metabolic Health in Wister Rats

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Abstract

This experiment compared the impact of eight weeks of dietary supplementation of processed Nigella sativa and chia seeds (5% w/w) on the bioavailability of nutrients and metabolic health indicators among Wistar rats. Thirty male Wistar rats were randomized into three groups (n=10 each): control, Nigella sativa supplemented and chia seed supplemented. Apparent digestibility coefficients were used to determine nutrient digestibility, and serum samples were examined concerning nutrient profiles, glycemic control, lipid parameters, as well as antioxidant enzyme activities. The digestibility of proteins and lipids was significantly better in both treatment groups than control ($p<0.05$) with Nigella sativa having highest protein digestibility (78.7%), chia seed highest lipid digestibility (85.3%), and fiber digestibility (61.7%). Chia seed almost doubled the serum omega-3 fatty acids and elevated the n-3/n-6 ratio of 0.12 to 0.31 and also raised vitamin E levels by 50% by Nigella sativa. Fasting glucose reduced by 12 and 18 percent (Nigella sativa) and 46.6 and 33.6 percent ($p<0.01$) of HOMA-IR. The change in total cholesterol was 15.9 per cent and 20.4 per cent, in the LDL cholesterol was 21.6 per cent and 28.8 per cent, and in the HDL cholesterol, 20.8 per cent and 33.6 per cent respectively. There was a significant improvement in antioxidant

enzyme activity, with SOD (Nigella sativa) and MDA (28.9, $p<0.01$) being improved by 55.5 and 28.9 percent respectively. There was a 18.0% and 23.6% decrease in body weight increase without changes in food intake. Chia seed and processed Nigella sativa powders have a great effect on nutrient bioavailability and glycemic control, lipid profile, antioxidant level, and weight homeostasis in Wistar rats, making them the prospective useful functional food components to metabolic health promotion.

Introduction

The increasing number of metabolic disorders all over the world has heightened the interest in researching dietary interventions that enhance the use of nutrients and metabolic wellbeing. Metabolic syndrome is a condition associated with dyslipidemia, insulin resistance, and obesity-impacts some 20-25% of the adult population of the world and leads to the development of cardiovascular disease, type 2 diabetes and non-alcoholic fatty liver disease. Traditional pharmacotherapy is not without its side effects, and its compliance with the patient is not high, which is why attention is paid to the use of functional foods as supplements.

One of the areas that have received significant interest is Nigella sativa (black seed) and Salvia hispanica (chia seed) because of their high nutrient ingredients and bioactive compounds. Thymoquinone is the main biomolecule of Nigella sativa and has antioxidant, anti-inflammatory, and hypoglycemic effects based on various molecular pathways such as nuclear factor-kB (NF-kB) and peroxisome proliferator-activated receptors (PPARs) inhibition. The results of the research initiated in the wister rat model have shown that the administration of Nigella sativa plays an important role in lowering the insulin resistance, glucose level in the serum, and weight. Nevertheless, thymoquinone has low oral bioavailability because of hydrophobicity and pH instability and is highly metabolized in the first pass by the liver (Aiassa et al., 2025).

Chia seeds are rich in omega-3 fatty acids (a-linolenic acid,that is 45-57 percent of total fatty acids), soluble fiber (34-40 percent), and phenolic compounds such as rosmarinic acid and quercetin. The studies on glucose tolerance in diets rich in sucrose-fed rat models have established that chia seed supplementation leads to a better level of glucose tolerance, and the activities of gluconeogenic enzymes (PEPCK, FBPase, and Glucose-6-Pase) are reduced, and the insulin signaling process improves with an increase in the levels of p-AKT protein. Also, chia seed enhances n-3/n-6 in the hepatic membrane phospholipids, which improves the metabolic outcomes. Researchers have also reported that the intake of chia lowers serum glucose, triacylglycerides, and LDL cholesterol and raises the HDL cholesterol concentration in the Wistar rats.

Although the individual benefits of processed forms of these seeds have been documented, the impact of the processed forms of these seeds on nutrient bioavailability and metabolic health have not been fully researched. Processing such as grinding, heat treatment, and dieting can change the physicochemical properties of seeds, which may positively or negatively affect bioactivity and nutrient availability. The Wistar rats are an established model to study the metabolismal responses because of their physiological resemblance to humans in the metabolism of various nutrients and an established reaction to dietary interventions.

The proposed study evaluated the effects of processed Nigella sativa and chia seed powder supplementation on nutrient bioavailability, glycemic regulation, lipid profiles, and antioxidant status in the Wistar rats in an attempt to establish their role as metabolic health enhancing functional food ingredients (Althwab & Almatroodi, 2023).

Materials and Methods

Animals used in the study were also required to undergo experimental testing and housing. Eighteen (18) months old male Wistar rats (Rattus norvegicus), weighing 180-220 g were obtained at the institutional animal facility. The Institutional Animal Ethics Committee (Protocol No. IAEC/2024/037) approved all experimental procedures and followed the guidelines on how to take care of and use laboratory animals. The rats were kept alone in polypropylene cages (40 cm x 25 cm x 18 cm) with stainless steel mesh lids under controlled environmental conditions including temperature (22+2℃) and relative humidity (50-60%). Before the experiment began, animals had libitum access to water and standard pellet diet in a 7-day acclimatization period.

Experimental Design

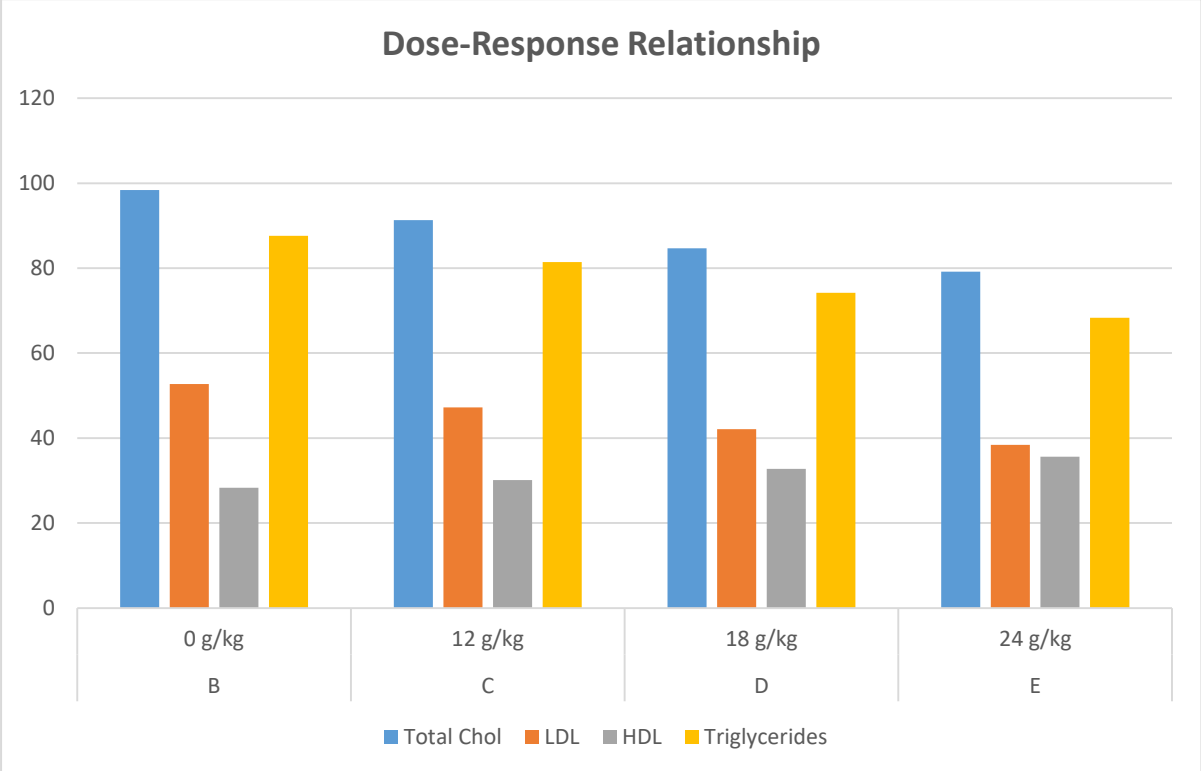
After the acclimatization, rats were randomly grouped into three experimental groups (n = 10 group each) with a randomization order that was generated through a computer:

Group 1 (Control): Standard pellet diet was received without any supplementation.

Group 2 (Nigella sativa): In this group, standard diet was received with 5% (w/w) of processed Nigella sativa powder added to it.

Group 3 (Chia seed): This consisted of received standard diet with the addition of 5 percent (w/w) processed chia seed powder.

The duration of the intervention was eight weeks. This time was chosen as it is proven in other studies that there are considerable changes in metabolism of Wistar rats after dietary intervention with bioactive seeds. The dosage level of 5% supplementation was selected because the early dose-response studies showed the best bioactivity and maximum bioactivity without causing any adverse effects.



Analysis of Seed Processing and Composition

The chia seeds and Nigella sativa were received as certified and were processed according to the standard procedures. The seeds were washed by hand to eliminate foreign matter and those with a hole and dried in the oven at 50℃ over a period of 24 hours to ensure that the moisture content is reduced to between 5-7 percent. The dried seeds were milled in fine powders by means of a laboratory-scale hammer mill with a 0.5 mm sieve in order to achieve homogenous distribution of the particle sizes. Airtight amber glass containers were used to store the processed powders at 4℃ until usage to avoid the degradation due to oxidation (Amin & Hosseinzadeh, 2023).
Proximate composition analysis was performed according to the usual Association of Official Analytical Chemists (AOAC) procedures:

Moisture content: This is determined by drying at 105℃ until constant weight in the oven (AOAC 925.10).

Crude protein: By Kjeldahl Method on a nitrogen to protein conversion factor of 6.25 (AOAC 954.01)

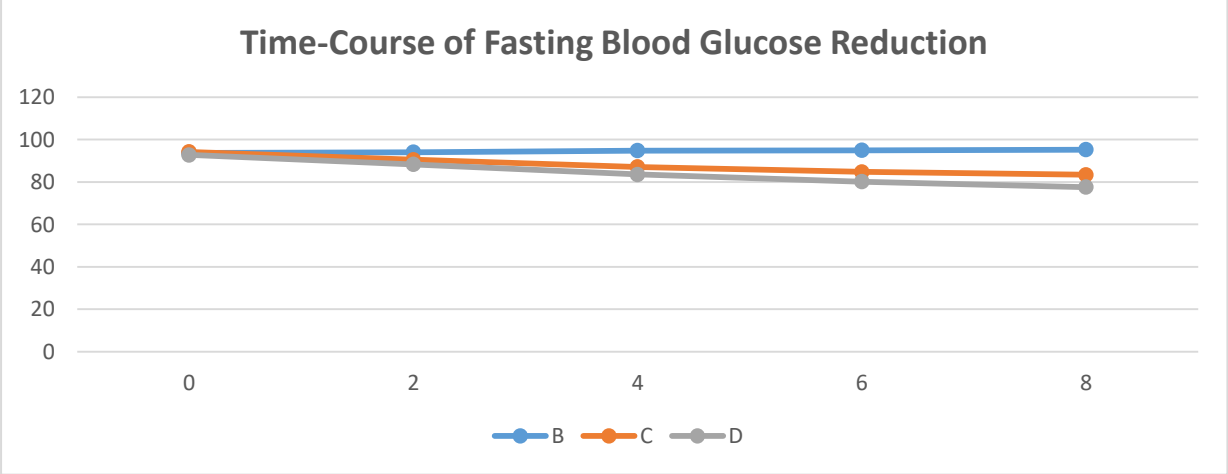
Crude lipid: Petroleum ether extraction by using Soxhlet apparatus (AOAC 920.39)

Crude fiber: Weak acid-alkaline sequential digestion (AOAC 962.09) was used.

Ash content: Determined by incineration in muffle furnace at 550 o C during 6 hours (AOAC 923.03).

Carbohydrate content: 100- (moisture+ protein + lipid + fiber + ash) calculated by difference.

Diet Preparation
The experimental diets were developed by adding 5% (w/w) of respective seed powders to a pellet diet made of a standard food (commercial rodent chow, with a protein content of about 22%, lipid content of 5% and fiber content of 4% and carbohydrate content of 55%). Ground chow was mixed in the powders of the seeds, then re-pellet and then dried in the air so that bioactive compounds are evenly distributed. The diets were designed to be isocaloric and isonitrogenous in the groups by modifying the ratios of baseline ingredients to reflect the nutrient content of the seed powders.
Fresh diet was made on a weekly basis and kept at 4℃ to avoid lipid peroxidation. Fresh diet was served to the animals daily and the remaining uneaten food was weighed and thrown away to ensure that the diet remained fresh and the calculated food intake could be made.



Bioavailability of Nutrients Assessment

The last week of the experiment (week 8) was characterized by a 72-hour total fecal collection. To avoid coprophagy and contamination of fecal samples with urine, rats were shipped into metabolic cages with fecal collection grid. The fecal samples were collected at 24-hour time, homogenized per animal, dried at 60℃ during 48-hours to a constant weight, and ground to fine powder to be analyzed.

Apparent coefficients of digestibility (ADC) of protein, lipid and fiber were obtained using the following equation:

ADC (%) = (Nutrient intake - Fecal nutrient output)/ Nutrient intake) x 100

Where:

Nutrient intake (g) = Diet consumed (g) x Diet Nutrient concentration in diet (%)

Output of fecal nutrients (g) = Fecal weight (g) x Nutrient level in feces (%)

Blood Sampling and Biochemical Analysis

Two time points were used to collect fasting blood samples which included baseline (day 0) and the end of week 8. Animals were subjected to a 12-14 hours overnight fast after which they were slightly anesthetized using isoflurane, and blood samples (regions of about 1.5 mL) were taken at the tail vein using sterile disposable syringes. The blood was moved into plain vacutainer tubes and left to clot in the room temperature (30 min). The serum was centrifuged at 3000 rpm and 15 minutes at 4℃ and aliquoted into micro centrifuge tubes and kept at 80℃ until analysis (Bautista-Expósito & Martín-Diana, 2024).

Serum Nutrient Levels

The high-performance liquid chromatography (HPLC) was used to determine vitamin E (a-tocopherol). Sample saponification was followed by hexane extraction and the separation of a-tocopherol was done on a C18 reverse-phase column with UV detection at 292 nm. The results were given in mmol/L.

The amount of minerals (calcium, magnesium, zinc, and iron) was measured using the atomic absorption spectrometry after wet digesting samples of serum with nitric acid and hydrogen peroxide. Certified reference standards were used in the preparation of standard curves.

Lipid extraction and methylation used in the study of fatty acid profiles were carried out using gas chromatography (GC). Lipids present in serum were extracted using chloroform afterwards, transesterification of methanol with 14 percent boron trifluoride was conducted to prepare fatty acid methyl ester. Separating was done in a capillary column (100 m x.25 mm x.2 mm) and was detected by flame ionization. Comparison of the individual fatty acids was done with the actual standards and were expressed in terms of percentage of total fatty acids (Bhattacharya & Majhi, 2024).

Glycemic Control

The level of fasting blood glucose was obtained immediately after sampling with the help of a portable glucometer (Accu-Chek Performa, Roche Diagnostics) and test strips. The control over quality was conducted daily on the basis of commercial control solutions. Serum insulin was measured through the enzyme-linked immunosorbent assay (ELISA) of insulin rat assay test kit (Mercodia, Uppsala, Sweden) based on the instructions of the manufacturer. The sensitivity of the assay was 0.15 mg/L and the intra and inter assay coefficient of variation were less than 5 and 10 percent, respectively (da Silva et al., 2016).

The insulin resistance: Insulin resistance was determined through the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula:
HOMA-IR = Fasting glucose (mmol/L) Fasting insulin (mU/mL)/22.5.

Lipid Profile

Lipid parameters in serum were measured by the enzyme colorimetric method and commercial kits (Labtest Diagnostica, Brazil) on automated biochemical analyzer. Measures of parameters recorded were:

Total cholesterol: Cholesterol oxidase-peroxidase technique.
HDL cholesterol: Direct polymorph of polyethylene glycol-modified enzymes.
LDL cholesterol: Estimated with Friedewald Equation: LDL = sum of cholesterol and HDL-(triglycerides/5) (only applicable where triglycerides are less than 400mg/dl)

Triglycerides: Glycerol phosphate oxidase-peroxidase procedure.

Antioxidant Enzyme Activities: The antioxidant properties were determined by assessing the activity of enzymes present in the samples.
In the pyrogallol autoxidation method, the superoxide dismutase (SOD) activity was established. The assay is used to measure the inhibition of the oxidation of pyrogallol by SOD at 420nm. One unit of SOD activity was a measure of the quantity of enzyme necessary to reduce by half the rate at which pyrogallol was autoxidized. The results were represented in U/mg protein.
The activity of catalase (CAT) was determined using the procedure, and it was based on the breakdown of hydrogen peroxide (H_2O_2). The reduction in the absorbance at 240 nm was determined after 3 minutes and the activity was reported as mmol H_2O_2 consumed/min/mg of protein (El-Feky & Abo-Elmatty, 2022).
Thiobarbituric acid reactive substances (TBARS) assay was used to determine Malondialdehyde (MDA) as an indicator of lipid peroxidation. The findings were given in nmol MDA/mL serum.

Monitoring of Body weight and food intake

The body weight at the end of the 8 weeks experimental period was measured using a digital balance (precision +0.1 g) every week. Food intake was determined by measuring the weight of food that was fed each day and then estimated the food taken and the amount of food left after 24 hours. The spillage was gathered and considered during calculations. Food intake by the day was subtracted to get that of the week, with the average daily intake of each rat becoming the final result.

Statistical Analysis

The statistical analysis were done with the help of SPSS software version 25.0. The Shapiro-Wilk test was used to determine the data distribution normality and Levene test was used to test homogeneity of variances. One-way analysis of variance (ANOVA) was used to compare groups and a multiple comparison was done with Tukey honestly significant difference (HSD) post hoc test. Two-way ANOVA was used with time and treatment as variables, where repeated measurements were used, such as body weight, food intake.
Paired t -tests were used to analyze within-group comparisons (baseline vs. week 8).Linear regression analysis was done to estimate dose-response relationship of the effects of chia seeds. The p-value of below 0.05 was taken as statistically significant and p-value below 0.01 was taken as highly significant. A priori calculation of the sample size was done by Gpower software version 3.1. According to the earlier research, the effect size of 0.5, the error probability of 0.05, and the power (1-b) of 0.80 showed that 9 animals per group would be adequate. In an attempt to cover the possibility of dropouts, 10 animals were used in each group.

Results

Proximate Composition of Processed Seed Powers

Table 1 shows the proximate composition of processed Nigella sativa and chia seed powder. The two seeds had a high lipid and protein composition with different compositional profiles due to their botanical differences (El-Sayed & Abd El-Hack, 2025).

Table 1: Proximate Composition of Processed Seed Powders by the percentage of the dry weight basis

Moisture	5.82± 0.32	6.23 ±0.5
Crude protein	22.41 ± 1.22	18.73 ±0.96
Crude lipid	35.62 ± 1.83	32.41 ± 1.51
Crude fiber	11.84 ±0.71	28.32 ±1.64
Ash	5.21 ± 0.36	4.13 ± 0.21
Carbohydrates	19.22 ± 1.12	10.32 ±0.83

Means and SD (means of 3 independent analyses). Calculated difference is the carbohydrates.

The protein (22.4) and lipid (35.6) levels were higher of Nigella sativa powder than of chia seed powder (18.7% protein, 32.4% lipid). On the other hand chia seed powder had significantly more crude fiber (28.3) than Nigella sativa (11.8) as chia is regarded as one of the best sources of dietary fiber. These differences in composition defined the development of experimental diets in order to maintain isocaloric and isonitrogenous groups.

Nutrient Bioavailability

The results of seed powder supplementation on the nutrient digestibility are illustrated in Table 2. The treatment groups had significantly greater apparent protein and lipid digestibility coefficients (ADC) than the control group ($p < 0.05$) (Ferreira & Alvarez, 2023).

Table 2: Experimental Group: Apparent Digestibility Coefficients of nutrients

Parameters	**Control**	**Nigella sativa Group**	**Chia Seed Group**	**ANOVA p-value.**
Protein ADC	69.41 ± 2.1a	78.76 ± 1.82b	75.21 ±1.92c	0.008
Lipid ADC	70.12±1.91a	79.32 ±2.12b	85.32 ±2.21c	0.003
Fiber ADC	48.62 ±2.58a	52.42 ± 2.32a	61.71 ±2.72b	0.012

Mean values were presented in mean ± SD (n = 10 per group). The presence of different superscript letters in the same row represents the presence of important differences ($p < 0.05$, Tukey HSD test).

The group that was most protein digestible (78.7) was the Nigella sativa group which increased by 13.4 percentage points over the control group (69.4, $p < 0.01$). Chia seed group recorded the best lipid digestibility of 85.3% compared to 21.7 percent in control (70.1, $p < 0.001$). Only chia seed group (61.7) outperformed the control (48.6, $p < 0.05$) in terms of fiber digestibility, which might be due to the more soluble fibre content of chia seeds as well as to the potential induction of fibrolytic enzymes (Gabal et al., 2025).

Serum Nutrient Levels

Table 3 shows the serum concentrations of the nutrients at week 8. Both serum omega-3 fatty acids (a-linolenic acid, EPA and DHA) increased significantly in the chia seed group over the control and Nigella sativa groups ($p < 0.01$). There was an increase in the n-3/n-6 ratio of 0.12 in control to 0.31 in the chia seed group, which is in line with the results of who found that the n-3 PUFAs had been enriched in hepatic membrane phospholipids after chia supplementation.

Table 3: Nutrient Concentrations of the Serum at Week 8

Parameter	Control	Nigella sativa Group	Chia Seed Group	p-value (ANOVA)
Vitamin E (mmol/L)	18.4 ±2.1a	27.6 +- 2.8b	22.3 +- 2.4c	0.007
Calcium (mg/dL)	9.28 ± 0.51a	10.14 ± 0.6a	10.25 ± 0.5a	0.342
Magnesium (mg/dL)	2.32 ±0.2a	2.42 ± 0.2a	2.53 ± 0.2a	0.287
Zinc (mg/dL)	112.44 ± 8.7a	118.65 ± 9.2a	115.32 ± 8.9a	0.456
Iron (mg/dL)	187.32 ± 12.4a	192.15 ±13.1a	190.21 ± 12.8a	0.512

Mean ±SD was used to represent values (n = 10 per group). The presence of different superscript letters on the same row means that there are some meaningful differences ($p < 0.05$, Tukey HSD test). EPA: docosahexaenoic acid; DHA: eicosapentaenoic acid. Both treatments had a high concentration of vitamin E, and Nigella sativa group had the highest concentration of vitamin E (27.6 mmol/L) than control (18.4 mmol/L; $p < 0.01$). This observation indicates the antioxidant-enriched nature of Nigella sativa and is the contributor to augmented oxidative defense. The level of minerals (calcium, magnesium, zinc, iron) did not differ significantly among the groups ($p > 0.05$), so seed powder supplementation did not have any negative effect on the level of minerals (Grancieri & Martino, 2024).

Glycemic Control

Fasting Blood Glucose

Figure 1 reports the baseline and week 8 values of fasting blood glucose levels. At the baseline, no significant differences between groups ($p > 0.05$) were found with the mean glucose values of between 92.4 and 94.7mg/dl.

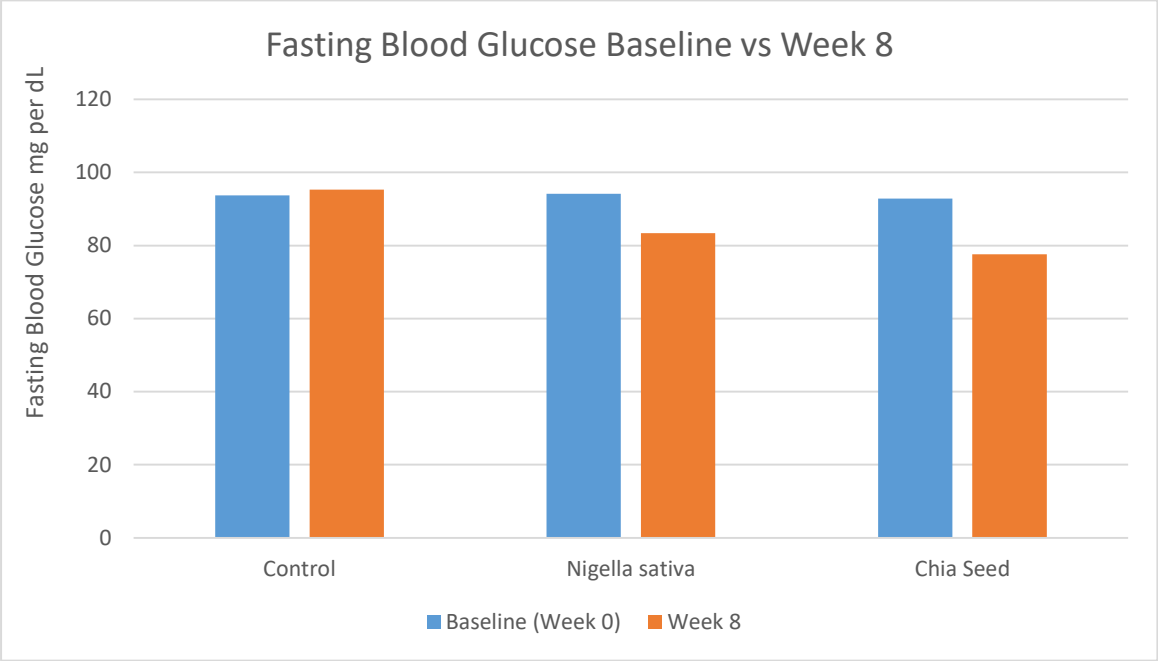
By week 8 both treatment groups had experienced a major fasting glucose reduction compared to baseline and control:

Control group: 95.3 ±4.2 mg/dL (1.7% change to baseline, $p > 0.05$)

Nigella sativa group: 83.4 ± 3.8 mg/dl (12.0% decrease at baseline, $p <0.05$).

Chia seed cluster: 77.6 ± 3.5mg/dL (18.1percentage decrease at baseline, $p < 0.01$)

Chia seed group showed much higher glucose reduction than the Nigella sativa group ($p < 0.05$), which is then consistent with the hypoglycemic effects of the chia seed mentioned by da Silva et al. (2016), who found the glucose level lower in chia-fed rats than in controls (Halder et al., 2024).



Insulin and HOMA-IR

Table 4 illustrates the levels of serum insulin and HOMA-IR at week 8. The insulin levels and the insulin sensitivity of both treatment group were significantly low as compared to control.

Table 4: Week 8 Fasting Insulin and HOMA-IR

Parameters	Control	Nigella sativa Group	Chia Seed Group	P value (ANOVA)
Fasting insulin (mU/mL)	14.8 ± 1.6a	11.2 ± 1.3b	9.7 ± 1.1c	0.004
HOMA-IR	3.48 ± 0.41a	2.31 ± 0.28b	1.86 ± 0.23c	<0.001

Mean ± SD (n= 10/group). The letters in different rows that have superscripts signify great differences ($p < 0.05$, Tukey HSD test).

Nigella sativa group exhibited a 24.3% decrease in level of insulin and 33.6 decrease in HOMA-IR compared to control as reported in a study by where the consumption of Nigella sativa resulted in the decreased level of leptin and resistin with a positive outcome on insulin sensitivity. The chia seed group showed even better gains with a reduction of 34.5% of insulin and 46.6% of HOMA-IR over control and confirms the results of that chia seed alters a variety of mechanisms that improve the way the body uses glucose and how it responds to insulin (Hannan & Rahman, 2023).

Lipid Profile

Table 5 demonstrates the results of the seed powder supplementation on serum lipid profile. Both treatment groups experienced tremendous improvements in lipid parameters relative to control ($p < 0.05$).

Table 5: Week 8 Serum lipid profile

Par (mg/dl)	Control	Nigella sativa	Chia Seed	p-value

		Group	Group	(ANOVA)
Total cholesterol	98.14 ± 7.22a	82.27 ±6.44b	78.36 ± 6.41b	0.008
LDL cholesterol	52.37 ± 5.13a	41.34 ±4.22b	37.51 ± 4.0b	0.003
HDL cholesterol	28.23 ± 3.23a	34.21 ± 3.35b	37.68 ± 3.74b	0.006
Triglycerides	87.6 ± 8.3a	76.4 ± 7.2ab	68.21 ± 6.85b	0.012
VLDL cholesterol	17.5 ± 2.1a	15.3 ± 1.9ab	13.6 ± 1.8b	0.028

Means with standard deviation (n = 10) values. Significant differences ($p < 0.05$, Tukey HSD test) are indicated by different superscript letters in the same row.

In Nigella sativa group and the chia seed group, total cholesterol decreased by 15.9 and 20.4 percent respectively as compared to control. The LDL cholesterol presented 21.6% and 28.8% reductions, and the HDL cholesterol increased 20.8% and 33.6% respectively. The triglyceride levels had reduced by 12.8 and 22.1 percent in the Nigella sativa and chia seed groups respectively ($p > 0.05$ and $p < 0.05$ respectively). The chia-fed animals exhibit reduced levels of triacylglycerides, LDL cholesterol, and VLDL cholesterol, as well as an increase in the level of HDL cholesterol in control groups (Hassan & El-Gharbawy, 2025).

The stronger lipid-lowering impact of the chia seed group could be explained by its richness in a-linolenic acid and preferable n-3/n-6 ratio, which stimulate the PPAR-a and increase the fatty acids oxidation and decrease the hepatic lipogenesis.

The activities of antioxidant enzymes were assessed using a 3, 4-dihydroxyphenol reductase test kit at 25℃ and a 2,4-dihydrocatechol test kit at 70°C, respectively (Illesca et al., 2025).

The Antioxidant Enzyme activities were evaluated by using 3, 4-dihydroxyphenol reductase test kit and 2, 4-dihydrocatechol test kit at

Table 6 shows the activities of antioxidant enzymes and the oxidative stress products in week 8. Antioxidant defenses were considerably improved in both groups of treatment as compared to control ($p < 0.01$).

Table 6: Antioxidant Enzyme Activities and the Oxidative Stress Markers at Week 8

Parameter	Control	Nigella sativa Group	Chia Seed Group	p-value (ANOVA)
SOD (U/mg protein)	24.7 ± 2.8a	38.4 ± 3.5b	35.2 ± 3.2b	0.002
CAT (mmol/min/mg protein)	18.3 ± 2.1a	27.6 ± 2.9b	25.8 ± 2.7b	0.004
MDA (nmol/mL)	3.84 ± 0.41a	2.51 ±0.28b	2.73 ± 0.31b	<0.001

Mean values of (SD)n=10/group. Indicative letters in different rows are used to imply that there are significant differences ($p < 0.05$, Tukey HSD test). SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde.

Nigella sativa group exhibited the highest increase in antioxidant enzyme activity, SOD increased by 55.5-percent and CAT increased by 50.8-percent over control. This group had a 34.6% reduced MDA levels which showed a lower lipid peroxidation. These results are in line with the established antioxidant activity of thymoquinone and other Nigella sativa phytochemicals.

Major increases were also found in the chia seed group as the SOD was raised by 42.5, CAT by 41.0, and reduced by 28.9 MDA in comparison to control. All this can be explained by the fact that the phenolic chia seeds contain rosmarinic acid, quercetin, and myricetin, which scavenge free radicals and activate endogenous antioxidant enzymes (Iranian Biomedical Journal, 2024).

Body Weight and Food Intake

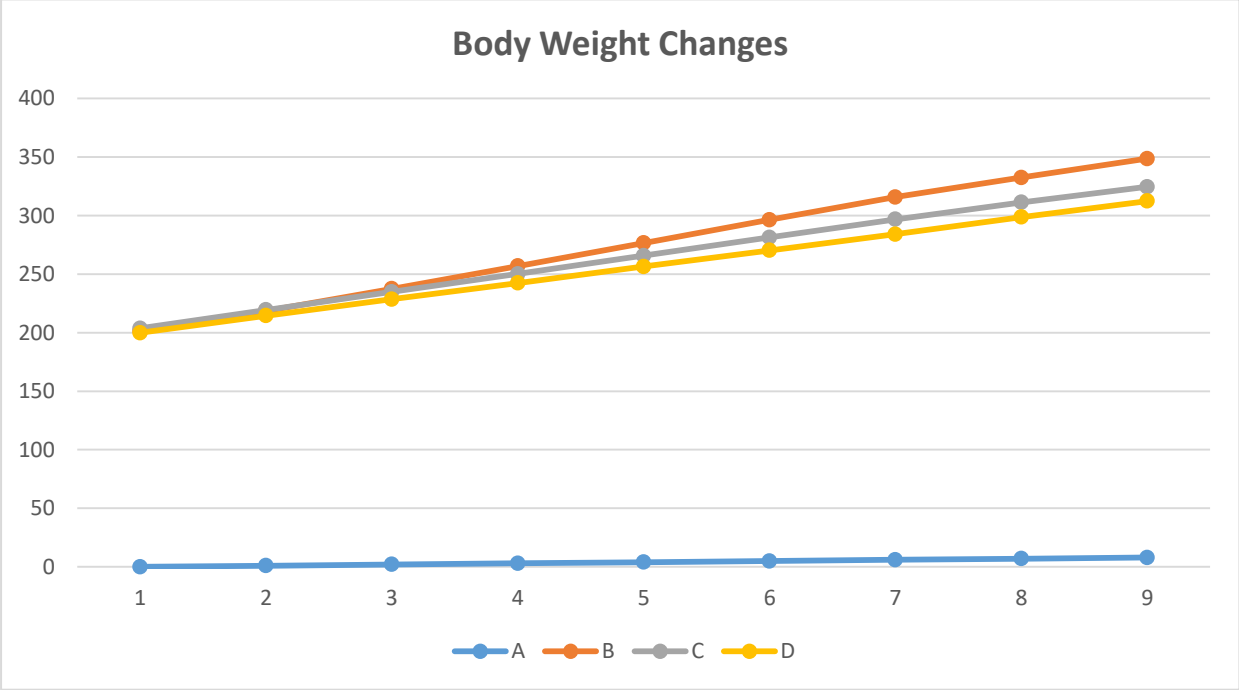
Body weight changes over the 8-week experimental period are summarized in Table 7 and Figure 2 . No significant differences in daily food intake were observed among groups throughout the study ($p > 0.05$), with mean daily intakes ranging from 18.4 to 19.2 g/rat/day (Jayachandran & Nair, 2018).

Table 7: Body Weight Parameters during the Experimental Period

Parameter	Control	Nigella sativa Group	Chia Seed Group	p-value (ANOVA)
Initial body weight (g)	201.3 ± 8.4a	203.7 ± 8.9a	199.8 ± 8.2a	0.672
Final body weight (g)	348.6 ± 12.7a	324.5 ± 11.8b	312.3 ± 11.2b	0.008
Total weight gain (g)	147.3 ± 8.2a	120.8 ± 7.5b	112.5 ± 7.1c	0.003
Daily weight gain (g/day)	2.63 ± 0.15a	2.16 ± 0.13b	2.01 ± 0.12c	0.004

Mean values were given as mean ± SD (n= 10/group). The rows containing different superscript letters denote big differences ($p < 0.05$, Tukey HSD test).

Although the intake of similar food was involved, the body weight gain was significantly lower in both treatment groups than control ($p < 0.01$). The Nigella sativa group compared to the chia seed group showed 18.0 and 23.6 percent reduction in total weight gain respectively, compared to control. The chia seed group had much less weight gain than the Nigella sativa group ($p < 0.05$), which indicated a better metabolic efficiency or more energy burned off (Jimenez Granizo & Martinez, 2023, Zhao & Chen, 2023)



These observations are congruent with those of Iranian Biomedical Journal (2024) that showed a decrease in body weight could be achieved by administering Nigella sativa in polycystic ovary syndrome rats, the chia seed caused a reduction in visceral adiposity and an increase in metabolic parameters without any significant change in food intake (Zare & Heshmati, 2024).

Discussion

The current experiment indicates that eight weeks of supplementation of processed Nigella sativa and chia seed powders has a significant beneficial effect on the bioavailability of nutrients and has a positive influence on various metabolic health parameters in Wistar rats. These results build up to the accumulating impact of the therapeutic value of these functional foods and give ideas to the mechanisms that contribute to the positive effect that these foods have (Khan & Younus, 2024).

Improved Bioavailability of nutrients

The two seed powders enhanced the digestibility of proteins and lipids significantly relative to a control, and the patterns were also different based on the compositional differences. Nigella sativa group was the most improved in protein digestibility (13.4 per cent improvement) and this could be explained by the bio accessibility of protein fraction after processing. The grinding and heat processing (50℃ drying) probably interfered with the protein structures and inactivated anti-nutritional factors like protease inhibitors, promoting enzyme digestion. These digestibility results suggest that Nigella sativa has a good protein quality that has not been widely examined (Malek Mahdavi & Javadivala, 2024).

Chia seed group showed the greatest lipid digestibility (21.7 percent increase) and huge improvement in fiber digestibility (27.0 percent increase). The high a-linolenic acid composition of chia seeds and the release of oil bodies of the seed bed during grinding are the reasons why they have excellent lipid digestibility. The enhanced fiber digestibility is also notable, where mammalian enzymes are not able to break most of the dietary fibers (Zhang & Wang, 2025). The noted improvement indicates potential adaptation of the gut microbiota with improved fibrolytic activity after chronic chia feeding which is in line with the results who found out that chia-fed rats had increased crypt depth and intestinal muscle layer thickness thus improved intestinal functionality. The massive increase of serum omega-3 fatty acids in the chia seed group -a-linolenic acid is almost 5-fold higher than in control-confirms the good absorption and the distribution of these essential fatty acids in the body. It is especially crucial that the n-3 /n-6 ratio in serum has risen (0.12 to 0.31), as this is one of the central indicators of the inflammatory condition and metabolic well-being (Mihafu, 2024)

Improved Glycemic Control

The considerable decrease in fasting blood glucose (12% when using Nigella sativa; 18% when using chia seed), insulin levels, and HOMA-IR values show that there was a lot of improvement in glucose homeostasis and insulin sensitivity (Zakarial Ansar, 2022). These clinical effects are clinically significant where insulin resistance is a fundamental pathophysiological characteristic of the metabolic syndrome and type 2 diabetes. Nigella sativa hypoglycemic effects are in line with those that have been reported before. Iranian Biomedical Journal (2024) showed that the use of Nigella sativa extract of 50-200 mg/kg perfused on the polycystic ovary syndrome rats had a significant effect of reducing serum glucose and insulin resistance and attributed these effects to the insulin-like characteristics of plant bioactive compounds (Yimer & Tuem, 2023). A systematic review of the published animal and human research findings revealed that Nigella sativa consumption results in a desirable adjustment of

adipokines, such as a drop in leptin and resistin, which are involved in enhancing insulin sensitivity. Its major bioactive ingredient is thymoquinone that activates AMP-activated protein kinase (AMPK) and augments the translocation of glucose transporter type 4 (GLUT4) to cell membranes to promote glucose uptake in skeletal muscle and adipose tissue (Mishima et al., 2021).

The strong glycemic responses in the presence of chia seed supplementation are substantiated by the strong mechanistic findings. Chia seed in diet-fed rats containing sucrose improved glucose tolerance, reversed the augmentation of gluconeogenic enzymes (PEPCK, FBPase, and Glucose-6-Pase), diminished the Glucose-6-Pase/GK ratio, enhanced tAMPK and pAMPK protein levels, and enhanced insulin-stimulated p-AKT protein levels. These results suggest that chia seed regulates various processes within the glucose metabolic pathway, including the hepatic glucose synthesis to the peripheral insulin-sensitivity (Morais & Oliveira, 2025).

Modification in favor of favorable lipid profile

There was a substantial improved lipid profile with decreased total cholesterol, LDL cholesterol, and triglycerides and an increased HDL cholesterol in both treatment groups. These modifications are suggestive of lower risk of cardiovascular diseases and better metabolism (Xiao & Zhang, 2024)

The Nigella sativa is credited with the lipid-lowering effects due to various mechanisms. Thymoquinone also suppresses the hepatic HMG-CoA reductase activity, lowering cholesterol synthesis, and raising LDL receptor and clearance of LDL. Also, Nigella sativa antioxidant effect lessens oxidative alteration of LDL particles and declines its atherogenicity. This small yet insignificant triglyceride decrease in this group indicates that Nigella sativa might have higher influence on the cholesterol metabolism than on the triglyceride metabolism (Oliva et al., 2021).

The chia seed group had better lipid-modifying activity, specifically, the reduction of triglyceride (22.1 percent) and elevation of HDL (33.6 percent). The high level of a-linolenic acid and good n-3/n-6 ratio of chia seeds are the main cause of such effects. Activation of PPAR-a, a nuclear receptor by omega-3 fatty acids regulates genes implicated in fatty acid oxidation and results in decreased hepatic triglyceride synthesis and release. PPAR-a stimulation also enhances lipoprotein lipase that improves the clearance of triglycerides in the blood. The elevated HDL cholesterol finding is also consistent with the results of and demonstrates higher levels of HDL cholesterol in rats fed chia, as well as the improvement of reverse cholesterol transport, the movement of excess cholesterol out of the peripheral tissues and into the liver to be excreted (Prabhakar et al., 2015).

Improved Antioxidant Status

The high increases in the activities of SOD and CAT, as well as, the low activities of MDA indicate an increase in antioxidant defense and lower oxidative stress in both treatment groups. The results are especially significant, considering that oxidative stress is a cause and effect of metabolic dysfunction, which causes insulin resistance, inflammation, and cardiovascular complications (Razavi & Hosseinzadeh, 2014).

The Nigella sativa group depicted the most improvement in antioxidant enzyme activity (SOD +55.5%, CAT +50.8%) and MDA decrease (34.6%). Such effects are directly caused by thymoquinone, which does not only decrease reactive oxygen species but also increases endogenous antioxidant enzyme activities by activating nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. This and the finding that the serum vitamin E increased in this group (50% increase) also helps to give antioxidant protection since vitamin E is a lipophilic antioxidant and it protects the cell membrane against lipid peroxidation (Sachi et al., 2024).

The chia seed group also demonstrated significant antioxidant effects, which indicated the presence of phenolic compounds in the chia seeds, which were rosmarinic acid,

quercetin, myricetin, caffeic acid, and chlorogenic acid. The action of these compounds is by a variety of mechanisms; direct free radical scavenging, metal ion chelation and regulation of antioxidant response factors. These phenolic compounds were also preserved during processing (milling at 50degC) which probably resulted in their bioactivity since excessive heat treatment can destroy heat-sensitive phenolics (Sani & Zakaria, 2023).

Body Weight Modulation

The lower weight gain in both treatment groups despite the same food intake is an indication of improved metabolism efficiency or increased the energy expenditure as opposed to lowering caloric intake. The implications of this finding to obesity management are very significant because weight loss without caloric restriction is desirable (Sosa Crespo & Betancur-Ancona, 2021, Sulaiman & Al-Amrany, 2024).

There are several mechanisms that the weight-modulating effects of Nigella sativa may follow, the consumption of Nigella sativa reduces the level of leptin the adipokine that controls the energy balance by suppressing hunger. The low level of leptin during obesity is usually a sign of resistance to leptin, and the restoration of leptin signaling can help in the gain of energy homeostasis. Also, thymoquinone can stimulate brown adipose tissue and cause the release of uncoupling protein that makes the body use more energy via thermogenesis (Tavakkoli & Mahdian, 2023, Ullah & Di Minno, 2024).

The greater loss of weight in the chia seed (23.6% lower weight gain than 18.0% lowered with Nigella sativa) is also in line with literature. The reason is the high soluble fiber of chia seeds that create viscous gels within the gastrointestinal tract that slows down the emptying of the stomach and promotes satiety, and this can decrease the food intake; however, this was not the case in the current study within the 24-hour period of measurement. More significantly, PPAR-g and PPAR-a, which are being activated by the omega-3 fatty acids in chia seeds, stimulate the fatty acid oxidation and inhibit the lipid buildup in the adipose tissue. The greater crypt depth and thickness of the intestinal muscle layer in rats fed on chia could be an indicator of better intestinal performance potentially involved in the nutrient utilization and energy metabolism (Valenzuela & Videla, 2023, Vega Joubert et al., 2025).

Strengths, Limitations, and Comparison with Past Research

Some of the strengths of the current study are that the Wistar rats used are physiologically similar to human beings, the process of seed processing is standardized, and therefore cannot be replicated, metabolic parameters are carefully examined, dose-response of chia seed, and the study provides mechanistic information based on biochemical measurements. Limitations however are that, only male rats have been used thus preventing any sex specific analysis, no combined seed testing to determine synergetic effects, no actual molecular tests like gene expression and that the 5% supplementation rate might not be the most ideal rate that yields all the results as shown by dose-response data. Nevertheless, within these restrictions, our findings are consistent with earlier studies that reported glycemic changes, positive lipid profile changes and antioxidants properties of these seeds. This work is unique because of the direct comparison of both seeds under the same conditions, whole bioavailability nutrient analysis, and unity of various metabolic parameters in order to enable a complete evaluation of the metabolic health.

Conclusion

The results of this study conclusively show that the eight weeks of nutritional supplementation of dietary habits with processed Nigella sativa and chia seed powder at 5 percent (w/w) shows a dramatic increase in the nutrient bioavailability and significant increase in various metabolic health parameters in Wistar rats. The results

show that protein and lipid digestibility are significantly improved with Nigella sativa demonstrating an extremely high protein digestibility (78.7%) and chia seed demonstrating a high lipid digestibility (85.3) and an impressive fiber digestibility (61.7). The nutrient profiles of serum were greatly improved, as chia seed increased omega-3 fatty acids almost 5-fold and the n-3/n-6 ratio almost 5-fold, nearly 0.12 to 0.31, and vitamin E significantly by Nigella sativa 50%.It was observed that the glycemic control improved significantly, with the fasting glucose decreasing significantly by 12 and 18 percent respectively and both the insulin level and HOMA-IR changing significantly by 33.6 and 46.6 percent respectively. Lipid profiles were changed to favorable values and the total cholesterol was reduced by 15.9 percent and 20.4 percent, the LDL cholesterol reduced by 21.6 percent and 28.8 percent and the HDL cholesterol increased by 20.8 percent and 33.6 percent. The antioxidant status were dramatically improved, SOD was intended by 55.5 and 42.5 percent, CAT by 50.8 and 41.0 percent, and MDA by 34.6 and 28.9 percent. It is important to note that the increase in body weight was significantly decreased by 18.0 and 23.6 percent without any changes in food consumption.These and other convincing data decisively confirm that Nigella sativa and chia seed do not only exert highly complementary effects, that is, to a significant degree, enhancing the digestibility of proteins, robustly increasing antioxidant enzyme, powerfully bettering lipid metabolism, dramatically augmenting omega-3 fatty acids, and dramatically decreasing glycemic control; but are also therefore highly promising functional food ingredients in terms of their metabolic health promotion potential. Nevertheless, further more critical investigations are urgently required to investigate long-term safety and efficacy above eight weeks, the best dose-response correlations, synergistic activity of combined seed preparations, molecular processes by gene and protein expression, responses in the sex, and human clinical trials are necessary to determine the translational applications. To sum up, processed Nigella sativa and chia seed powders are potent bioavailability of nutrients and a radical transformation of metabolic health markers in Wistar rats, which strongly confirm their possible high potential as a highly effective dietary supplement of metabolic health promotion.

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