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SYNERGISTIC HYPOCHOLESTEROLEMIC AND HEPATOPROTECTIVE EFFECTS OF COMBINED CHIA (SALVIA HISPANICA) AND BASIL (OCIMUM BASILICUM) SEED POWDER IN A POLOXAMER-407 INDUCED HYPERCHOLESTEROLEMIC RAT MODEL

Bareera Ghaffar[*1], Aliha Rifat Awan[2], Umrah Zafar[3], Dua Ayesha[4], Ayman Latif[5], Rana Maira Majid[6], Warda Mahmood[7], Hafsa Ahmad[8], Arfa Mahmood[9], Abeeha Tareen[10], Muahammad Faisal[11], Muazammil Qazi[12]

[*1,3,4,6,10]*Department of Human Nutrition and Dietetics Muhammad Nawaz Sharif University of Agriculture, Multan*
[2,5]*Multan College of Food and Nutritional Sciences Project of MMDC, Multan*
[7,8,9]*Department of Nutrition and Dietetics Nishtar Medical University, Multan*
[11]*Department of Pharmacy Superior University, Lahore*
[12]*Department of Plant Protection of Pathology Muhammad Nawaz Sharif University of Agriculture, Multan*

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Corresponding Author: *
Bareera Ghaffar

Abstract

Hypercholesterolemia is a critical metabolic disorder characterized by elevated blood lipids and is a primary risk factor for atherosclerosis and cardiovascular diseases. While statins are effective, their side effects highlight the need for natural, dietary interventions. Chia (Salvia hispanica) and basil (Ocimum basilicum) seeds are rich in bioactive compounds like dietary fiber, omega-3 fatty acids, and polyphenols, which are known to modulate lipid metabolism. This study aimed to evaluate the hypocholesterolemic, hematological, and hepatoprotective effects of dietary supplementation with chia and basil seed powders, both individually and in combination, a poloxamer-407-induced hyperlipidemic rat model. Proximate analysis confirmed the high nutritional value of the seeds, with basil seeds exhibiting superior antioxidant activity (Total Phenolic Content: 18.31 ± 0.58 mg GAE/g; DPPH scavenging: 65.89 ± 2.07%) compared to chia seeds (TPC: 14.72 ± 0.42 mg GAE/g; DPPH: 58.14 ± 1.62%). Twenty male albino rats were divided into five groups: a normal control (T0), a hyperlipidemic control (T1), and three treatment groups receiving a high-cholesterol diet supplemented with either 2g chia seed powder (T2), 2g basil seed powder (T3), or a 2g chia + 2g basil seed powder combination (T4) for 28 days. The combined treatment (T4) demonstrated the most significant therapeutic effects. It markedly improved the lipid profile, reducing total cholesterol to 95.51 ± 1.9 mg/dL, triglycerides to 117.8 ± 1.6 mg/dL, LDL to 42.67 ± 1.2 mg/dL, and VLDL to 28.4 ± 1.1 mg/dL, while increasing HDL to 50.5 ± 1.0 mg/dL, compared to the hyperlipidemic control (T1). The seed powders also exhibited hepatoprotective properties, as evidenced by the significant reduction in liver enzymes ALT (42.2 ± 1.6 U/L), AST (58.2 ± 1.6 U/L), and ALP (39.5 ± 1.6 U/L) in the T4 group. Furthermore, supplementation preserved hematological parameters, maintaining hemoglobin (13.7 ± 0.4 g/dL), RBC count (8.4 ± 1.08 ×10³/μL), and hematocrit (37.4 ± 1.0%) near normal levels, and showed a

modulatory effect on WBC count (9.3 ± 0.2 ×10³/μL). All data were statistically significant at p < 0.05. In conclusion, the dietary incorporation of chia and basil seed powders, particularly in combination, demonstrated potent hypocholesterolemic, hepatoprotective, and hematopoiesis-supporting effects. These findings position the chia-basil seed mixture as a promising, natural dietary strategy for managing hypercholesterolemia and mitigating associated cardiovascular risks.

INTRODUCTION

Hypercholesterolemia is a complicated metabolic condition that produces a higher amount of lipids, in particular cholesterol and triglycerides, in blood. Such abnormality in
lipids is a major factor in the etiology and development of heart related illnesses (CVDS). Cholesterol is essential to normal physiologic functions including: maintaining the integrity of cell membrane structure, acting as pro-hormone to the production of steroid (e.g., cortisol, estrogen, testosterone), and bile acid synthesis (an essential process in the digestion of fat). When, however, cholesterol exceeds normal physiological levels, and is of much concern when it is more than 200 mg/dL in serum, it may penetrate the endothelial lining of arteries. This causes a sequence of pathological events resulting to the development of atherosclerotic plaques. These plaques cause the narrowing of the lumens of arteries, deform blood flow, and may rupture to form thrombosis and result in ischemic events; myocardial infarction or stroke (Brooks *et al.*,2019).

Primary hypercholesterolemia is as a result of gene defects inherited due to mutations in the major proteins that are actively involved in lipid metabolism, especially those in charge of clearing the LDL levels in the blood. The most important causes of primary hypercholesterolemia involve mutations in the LDL receptor gene (LDLR) and apolipoprotein B gene (APOB) as well as proprotein convertase subtilisin/kexin type 9 gene (PCSK9). These are related to impaired LDL receptors, impaired or inappropriate LDL particles clearance and resulting increase in cholesterol in circulation (Nordestgaard *et al.*, 2020).

The chia seeds are also rich component of vital micronutrients which are crucial to metabolic as well as skeletal and cardiovascular
wellbeing.Soluble fiber is one of the most important elements of chia seeds that causes cholesterol-lowering effects. Chia seeds are the richest dietary fiber source with about 34 to 40 percent of their dried weight in terms of fiber whereof a potent segment is soluble. Soluble fiber is one of the most important elements of chia seeds that causes cholesterol-lowering effects. Basil seeds also offer useful polyunsaturated fatty acids (PUFAs) that also include the crucial omega-3 fatty acid which is referred to as alpha-linolenic acid (ALA). ALA is known by the aspects of being anti-inflammatory and producing cardiovascular protection .Basil seeds also offer useful polyunsaturated fatty acids (PUFAs) that also include the crucial omega-3 fatty acid which is referred to as alpha-linolenic acid (ALA). ALA is known by the aspects of being anti-inflammatory and producing cardiovascular protection.

Materials and Methods

The study was carried out in the Central Hi-Tech Laboratory of MNS-University
of Agriculture, Multan.

2.2.1. Procurement of Samples

Chia and Basil Seeds were purchased from the local market of Multan usingcertified organic suppliers. Analysis chemicals and reagents of an analytical grade wereobtained at BDH, Sigma Aldrich, and Merck.

2.2.2. Seed Cleaning and Drying

Chia and Basil Seeds were carefully cleaned so as to eliminate dirt and foreign

materials. To further facilitate milling, seeds were then placed under the hot air oven
under a controlled temperature of 60oC/24hrs in an attempt to dry up the moisture content
in the seeds.

2.2.3. Grinding and Sifting

The study was carried out in the Central Hi-Tech Laboratory of MNS-University
of Agriculture, Multan.

2.2.4. Compositional Analysis of Basil and Chia seeds

Proximate composition of the seed's moisture, crude fat, crude fiber and ash were
determined according to description in AACC (2000).

Moisture content determines food quality, stability, and shelf life by affecting microbial growth and enzyme activity.It was measured in chia and basil seeds using the dry heat oven method.Samples (~5 g) were dried at 105°C for 6 hours, then cooled in a desiccator.

2.2.4.1 Moisture content was calculated from weight loss using the standard formula .Moisture content determines food quality, stability, and shelf life by affecting microbial growth and enzyme activity.It was measured in chia and basil seeds using the dry heat oven method.Samples (~5 g) were dried at 105°C for 6 hours, then cooled in a desiccator.Moisture content was calculated from weight loss using the standard formula.

Moisture Content (%) = (Initial Weight−Final Weight) × 100 / Initial Weight

2.2.4.2. Crude Fat Content

Fat content was determined using Soxhlet extraction method (AACC Method 30- 25) with petroleum ether as solvent. A pre-dried sample (5-6g) was weighed, wrapped in filter paper (thimble), and placed in the Soxhlet apparatus. After extraction and drying, fat content was calculated using the standard formula:

Fat % = (W1 - W2) / W1 × 100

Where:

W1 = Initial sample weight W2 = Weight after fat removal 29

2.2.4.4. Ash Content

Ash content was determined following AACC Method 08-01. Samples were weighed and charred using a spirit lamp, then ashed in a muffle furnace at 550–650°C for 5–6 hours. After cooling, ash percentage was calculated:

Ash % = W2 / W1 × 100

W1 = Initial weight W2 = Weight of ash

Total phenolic content (TPC)

The total phenolic content (TPC) of chia and basil seeds was determined using the
Folin-Ciocalteu method as described by (Tiwari *et al.*, 2011), with slight modifications. For extraction, methanol and HCl were mixed in a ratio of 90:8.2. A 5 mL aliquot of this extraction mixture was added to 1 g of finely ground seed sample. The sample was homogenized and then centrifuged at 9000 rpm for 15 minutes at 4 °C**.** From the supernatant, 300 µL was taken and mixed with 600 µL of 10% Folin
Ciocalteu reagent. Then, 2.4 mL of 7% Na_2 CO_3 solution was added to the mixture. The reaction mixture was incubated at room temperature for 30 minutes in the dark.

Afterwards, 200 µL of the final reaction mixture was transferred to a 96-well ELISA microplate, and absorbance was measured at 765 nm using a spectrophotometer. The TPC was calculated using a Gallic Acid Standard Curve and expressed as mg Gallic Acid Equivalents (GAE) per gram of sample using the following formula:

Phenol = (Sample C) DF / M30

Where C is the concentration of the sample, DF is the Dilution factor, and M is
the Extract weight.

2.2.4.6. DPPH Assay

The antioxidant activity of chia and basil seeds was measured by the 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay, following the method of (Tiwari *et al.*, 2011). A volume of 50 µL of the previously prepared methanolic extract was mixed with 5 mL of 0.004% DPPH solution in a 96-well microplate. The reaction mixture was allowed to incubate at room temperature in the dark for 30 minutes. Absorbance was measured at 517 nm using an Epoch ELISA Reader

(BioTek Instruments, Inc., USA). A blank consisting of methanol and DPPH solution was used. The DPPH free radical scavenging activity was calculated using the following formula:

DPPH % = $((A_0 - A_1) / A_0) \times 100$

2.3. Statistical Analysis

All measurements were conducted in triplicates. Results were expressed as mean ± standard deviation. Descriptive statistics were applied for variability and accuracy, as recommended by Montgomery (2017).

2.4. Results

Table 2.1: Proximate Composition of Chia and Basil Seeds

Components	Chia Seeds (%)	Basil Seeds (%)
Moisture content	6.12 ± 0.18	7.45 ± 0.23
Crude fat	30.45 ± 0.35	25.17 ± 0.41
Crude fiber	34.65 ± 0.28	21.36 ± 0.33
Ash content	4.92 ± 0.21	5.76 ± 0.18

Table 2.2: Total Phenolic Content (TPC) and DPPH Scavenging Activity of Chia and Basil Seeds

Parameter	Chia Seeds	Basil Seeds
TPC (mg GAE/g)	14.72 ± 0.42	18.31 ± 0.58
DPPH (%)	58.14 ± 1.62	65.89 ± 2.07

2.4.1. Moisture Content

The moisture content of chia and basil seeds was measured using the dry heat oven method, and results are presented in Table 3.1. Chia seeds had a moisture content of 6.12 ± 0.18%, while basil seeds showed 7.45 ± 0.23%. These results are within the acceptable limits for oilseeds, which generally maintain moisture levels below 10% to ensure shelf stability and minimize microbial growth. Similar values have been reported by Ayerza and Coates (2005), indicating that chia seeds possess relatively low moisture content suitable for long-term storage.

2.4.2. Crude Fat Content

The crude fat content of chia and basil seeds differed significantly. As shown in Table 2.1, chia seeds contained 30.45 ± 0.35% fat, whereas basil seeds had 25.17 ± 0.41%. . The higher fat content in chia seeds is attributed to their rich omega-3 fatty acid profile, particularly α-linolenic acid (ALA), which has been widely reported in literature (Mohd Ali *et al.*, 2012). In contrast, basil seeds, though also lipid-rich, contain slightly lower fat, which may reflect differences in their botanical structure and oil deposition.

2.4.3. Crude Fiber Content

Chia seeds were found to be rich in dietary fiber, with values reaching 34.65 ± 0.28%, while basil seeds contained 21.36 ± 0.33% (Table 2.1). These findings are consistent with those reported by Reyes-Caudillo *et al.*, (2008), who found fiber levels in chia as high as 32–34%. The significantly high fiber content in both seeds supports their potential application in

gastrointestinal health and blood sugar regulation. Dietary fiber not only promotes digestive well-being but also plays a role in cholesterol reduction, making these seeds beneficial in functional food formulations.

2.4.4. Ash Content

The ash content constitutes total mineral content of any food sample. In the present study, ash content in chia seed was 4.92 ± 0.21 and 5.76 ± 0.18 in basil seeds (Table 2.1). The values indicate that there is a rich proportion of essential minerals like calcium, phosphorus and potassium. Basil seeds having the presence of more ash can have more mineral-plussed health benefits. These findings have been supported in previous research by Ullah and others, (2016) that chia is a source of minerals with a lot of potential as a component of diets.32

2.4.5. Total Phenolic Content (TPC)

Antioxidant capacity of plant materials is an important indication that is indicated by total phenolic content (TPC). TPC in the TPC of chia and basil seed was measured by Folin-Ciocalteu method and quoted as mg gallic acid equivalents (GAE) per g dry weight. Table 2.2 illustrates that the content of phenol was greater in the basil seeds (18.31 ± 0.58 mg GAE/g) as compared to its counterpart, chia seeds (14.72 ± 0.42 mg GAE/g). The greater TPC of basil seeds shows a higher possibility of neutralizing free radicals. This finding can be compared to the results by (Rababah *et al.*, 2004) which proves the good phenolic composition of basil seeds.

2.4.6. DPPH Assay (Enzymatic Activity)

The antioxidant activity was measured in chia and basil seed extracts using DPPH radical scavenging assay. The findings (Table 2.2) revealed that basil seeds exhibited the greatest DPPH inhibition of 65.89 ± 2.07 when compared to those of chia seeds 58.14 ± 1.62. Such findings add credence to the idea that both seeds are good sources of natural antioxidants, the seeds of basil having a higher radical scavenging ability. This could also be attributed to fact that they contain more phenolics. These results also correspond to previous studies (Taga *et al.*, 1984) that phenolic rich foods are beneficial in oxidative stress reduction.

2.5 Discussion

2.5.1 Composition of Chia and Basil Seeds

The proximate composition of chia and basil seed shows clearly that there is a variety of differences in terms of their nutritional value and they contain varied dietary values. Moisture percentage is a very crucial factor towards determination of shelf stability and microbial safety of the seeds. In the present work, seeds of basil were found with a slightly better moisture level (7.45 ± 0.23%) in comparison to chia seeds (6.12 ± 0.18%). The two values are below the limit of 10 percent recommended to be below that limit as far as oilseed is concerned and they make them acceptable and perfectly suitable to be stored in the long term, without much loss to quality. A relatively low percentage of moisture in chia seeds might increase storage stabilityand oxidative preservation of these seeds with time. In terms of crude fat contents, chia seeds proved to have a large lipid concentration (30.45 ± 0.35) as compared to basil seeds (25.17 ± 0.41). This is in line with the documented nutritional composition of the chia seeds as a good source of omega-3 fatty acids, especially the alpha-linolenic acid (ALA), which plays a role in the maintenance of cardiovascular health. This minor difference in the fat percentage of basil seeds can be interpreted in terms of structure of the oil deposition and fatty acid composition but they can be noted as being rich in fats, and they are helpful in maintaining lipid levels through diet-based lipid control. A marked difference was reported in crude fiber where chia seeds had 34.65 ± 0.28% that was much higher than that of basil seeds which has 21.36 ± 0.33%.

The high concentration of fiber in chia substantiates its image as a digestive-health functional ingredient, glycemic regulator, and cholesterol regulator. Fiber content of both seeds is, however, quite high and they could be used as future components of dietary fiber addition plans and development of various functional foods. Basil seeds had 5.76 ± 0.18 percent more ash

than the chia seeds which amounted to 4.92 ± 0.21 percent.

The ash content provides evidence of the total mineral content, so it is possible that basil seeds provide slightly wider range of minerals including more calcium, magnesium and potassium. They are crucial in the metabolism and physiological ample processes like electrolyte balance, bone health, and enzyme actions in the body.

In antioxidant potential the Total Phenolic Content (TPC) and DPPH free radical scavenging activity revealed that that basil seeds had better antioxidant properties. TPC was lower in chia seeds (14.72 ± 0.42 mg GAE/g) than in basil seeds (18.31 ± 0.58 mg GAE/g), and thus it has a less-diverse phenolic content. This was enhanced by the DPPH findings where garam masala exhibited 65.89 ± 2.07 percent scavenging activities compared to 58.14 ± 1.62 percent by chia. These results recommend that basil seeds could be a valuable source regarding protection against oxidative stress that has been promoted in the pathogenesis of several forms of chronic disorders among them as cardiovascular illnesses, diabetes, and cancer.

EXPERIMENT-II: EFFICACY STUDY

3.1. Introduction

The present study work was carried out to study efficacy of chia (*Salvia hispanica*) and basil (*Ocimum basilicum*) seed powder in treatment of hypercholesterolemia with the help of experimental model upon albino male rats. Rat as a model rather than human subject made it possible to conduct an experimental animal setting that was controlled in terms of diet, equivalent environmental conditions, and efficient monitoring, hence ensuring the result consistency and reproducibility.This study was primarily carried out to determine the individual or combined effects of chia and basil seed powder on the parameters of the lipid profile in hypercholesterolemic rats. In this case, since there has been reported nutraceutical and functional food benefits, it was conceived that the seeds hold hypocholesterolemic potential, and thus can be used as a reasonable dietary means applicable in the management of high cholesterol levels. Their efficacy was evaluated by means of violation of a complex of biochemical and hematological parameters by means of experimental simulation and statistical analysis.

Chia seeds contain high levels of omega-3 fatty acids, dietary fiber, proteins, and polyphenols that all make them lipid reducing foods. Basil seeds, too, have some valuable proportions of fiber, essential fatty acids, and antioxidants that can facilitate cardiovascular functions and help decrease serum cholesterol. The article that is under discussion introduces an efficacy trial that aims at investigating such effects via well organized experimentation and results analysis. (Nadeem *et al.*, 2022) conducted a study and found out that chia seeds are composed of about 16-20 percent protein, 25-34 percent oil (of which over 60 percent is alpha-linolenic acid), 34-40 percent dietary fiber, and several essential minerals. The composition of fatty acids in chia was also mentioned in another paper by Ayerza and Coates (2021),where the chia contains 59 percent α-linolenic acid and makes its lipid-lowering effect possible.

In the same line of thought, (Ahmad *et al.*, 2020) found that basil seeds can be used to improve cardiovascular health because they are antioxidant and anti-inflammatory agents.

3.2. MATERIALS AND METHODS

3.2.1. Purchasing of raw material

Chia seeds (*Salvia hispanica*) and basil seeds (*Ocimum basilicum*) were purchased from the local market of Multan. The seeds have been washed with tap water to clean them by getting rid of dust and other impurities and then dried using air at the ambient temperature (25-30oC). After being dried fully, the seeds were crushed into a fine powder by the help of an electric grinder and sieved in the form of equal sized particles through 100-mesh sieve. The products of chia and basil seed powders separately packed, airtight and labeled were put in dry conditions awaiting a later use.

3.2.2. Reagents and Chemicals
Analytical-grade reagents such as distilled water, Poloxamer and ketamine were used throughout the study. All reagents were obtained from recognized chemical suppliers and used without further purification.

3.2.3. Ethical Consideration
All experimental procedures were carried out in accordance with the ethical standards of the Institutional Animal Ethics Committee of Muhammad Nawaz Sharif University of Agriculture, Multan. Approval was obtained prior to experimentation. Animal handling and procedures adhered strictly to the institutional guidelines for the care and use of laboratory animals.

3.2.4. Animal Housing and Selection Criteria
Twenty healthy male adult Wistar albino rats (200 ± 10 g) were purchased from a certified breeder in Multan and housed in the Animal House of the Department of Human Nutrition and Dietetics, Bahauddin Zakariya University, Multan. Rats were individually housed in stainless steel cages under controlled environmental conditions (temperature: 25–30°C; light/dark cycle: 12/12 hours). All rats were allowed to acclimatize for one week before the experiment began.

3.2.5. Induction of Hypercholesterolemia
Hypercholesterolemia was induced in the rats (except for the control group) by administering a poloxamer 407 injection dissolved in saline glucose solution alongside a basal diet for three consecutive weeks, following the model described by Al-Shehri (2012). A systematic review highlighted that both 0.5 and 1 g/kg IP doses of P-407 are effective in inducing hyperlipidemia in rats, with observed increases in total cholesterol, triglycerides, and LDL levels, along with decreases in HDL. The induction was a confirmed by evaluating serum lipid profiles at the end of the third week.

3.2.6. Feed Preparation
Feed formulations were prepared according to the method described by (Adeyemi *et al.*, 2017) with slight modifications. The diets for treatment groups were supplemented with varying ratios of chia and basil seed powders, as shown below , as shown below:

Groups	Normal Diet	Chia seeds (g/20g)	Basil seeds (g/20g)
(non-induced)	20g	-	-
T1 (induced)	20g	-	-
T2	18	2	-
T3	18	-	2

3.2.7. Experimental design
Rats were selected randomly and set in groups of five and exposed to theexperimental conditions described below by feeding a basal diet:
To (Negative Control Group): It was used as the normal control group. No treatment was applied to rats or they were not induced to have hypercholesterolemia.
T1 (Positive Control Group): In this group, hypercholesterolemia was induced by the use Of Poloxamer 407 but nothing was treated to the diet.
T2-T4 (Treatment) Groups: They were subjected to hypercholesterolemia induced by Poloxamer 407, then subjected to dietary regimens that include the following:

T2: Obtained a diet with chia seed powder.
T3: Received a diet supplemented with basil seed powder.

T4: A diet supplemented by combination of chia and basil seed powders.

The experiment was done in 28 days. 25g of feed per kilogram of body weight was given on a daily basis to all the rats. Physical examination was done once in a week and body weights and feed intake were observed. The blood samples of all the rats were collected on 0th, 14th and 28th days to determine the hematological and biochemical indicators of lipid profiles such as the total cholesterol, triglyceride, LDL and HDL levels.

3.2.8. Feed and water intake

The daily feed intake was quantified by deducting the amount of leftover feed from the total amount of feed consumed and measured in grams (g/day). The daily consumption of water was measured by graduated bottles and the intake of each group was noted. For monitoring any subsidiary effects of treatments on feed intake, the weight of feed intake was recorded regularly every week.

3.2.9. Body weight

Before the initiation and after each week of experiment, the weight of the animals and the weight of their feed intake was measured in grams per animal and compared to the weight reported in the first week.

3.3. Hematological parameters

On day 0, day 14, and day 28, the rats had a period of fasting of 12-16 hours before being slaughtered. The subjects were rendered unconscious by administering 0.3 ml of ketamine and using surgical blade no. 24; they were subsequently anesthetized. Then, blood was taken in containers containing non-coagulating gel and EDTA. Centrifugation of gel tubes at 1000 rpm for 15 minutes at 25°C to separate the serum. The serum that had been separated was collected in tubes named as Eppendorf and stored at - 20°C temperature in a freezer until it was ready to be analyzed biochemically. The examination of hematological parameters was conducted using blood in the EDTA vials, whereas the analysis of biochemical parameters was conducted using serum.

3.3.1.2. Hematocrit (HCT)

Blood sample is referred to as the aggregate character of erythrocytes volume with respect to aggregate volume of blood and the unit is percentage. The blood was sucked and pipetted into a micro hematocrit tube, up to deep thirds of the volume. This was followed by sealing of the tube with a sealer. Hematocrit tube was then centrifuged in hematocrit centrifuge at 2000 rpm within five minutes. This value was read after placing it in a Hematocrit reader and then recorded.

3.3.1.3. Red blood cells (RBCs)

The sample of blood was taken with a pipette in a red cell blood-counting marked up to 0.5 mark. Some yellowish liquid was introduced and the solution diluted to point 101. Pipette shaking was done. The result was calculated through the correct equipment of percentage. The counting chamber was filled with the blood through the pipette with the inclination of 45o angle. The hemocytometer was thereafter left to rest a time interval of 2-3 minutes so that the red blood cells may settle. The fixed red blood cells were counted subsequently.

3.3.1.4. White Blood Cells (WBC)

Blood was collected using a WBC pipette up to the 0.5 mark. The WBC dilution fluid was poured up to the 11th mark without delay. The pipette was spun horizontally between the thumb and fingers. A 1:20 dilution was achieved. The hemocytometer was cleaned, including the cover slip and the counting chamber. The cover slip was positioned onto the counting chamber by exerting a mild force. The liquid in the pipette was discharged at a 45-degree angle. The hemocytometer was allowed to rest for a period of 2-3 minutes in order to allow the cells to settle down. The white blood cells (WBC) were enumerated in the four prominent squares located at the corners of the counting chamber which contain a total of 16 smaller squares.

3.3.1.5. Platelets

Platelet count was achieved by introducing a small quantity of blood that has been diluted in a counting chamber called hemocytometer with ammonium oxalate a red cell lysing agent were then distinguished from other blood cells and counted. The count acquired from the hemocytometer is adjusted according to the dilution factor.

3.4. Biochemical parameters

The biochemical parameters consist of lipid profile renal and liver function test (creatinine, urea, bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase). To examine the effect of communion and fenugreek powder on liver and kidney of rats in terms of reducing cholesterol levels, the experimental procedures outlined in the Laboratory Procedure Manual for Biochemistry Profile were followed (NHANES, 2000).

3.4.1. Liver Function Test (LFT)

Assessment was conducted on liver function tests which included bilirubin, ALT, AST and ALP levels were quantified using the calorimeter method following the principles of (NHANES, 2000).

3.4.1.1. Alanine Amino Transferase (ALT)

The quantification of serum ALT levels was conducted using calorimetry method employed for its accuracy and sensitivity. Individual distinct tubes labeled as blank, sample and standard were meticulously prepared to ensure the reliability of the analysis. 500 µl of the chromogenic reagent was added to each tube and combined with 100 µl of the respective solution blank, sample or standard. An additional 5ml NaOH is used to precisely quantify ALT.

Well, was added in tube. Subsequently all mixture were allowed to stabilize at room temperature for a duration of 15 minutes to facilitate proper reaction. The absorbance of the sample solution was meticulously compared to that of the standard solution.

3.4.1.2. Aspartate Amino Transferase (AST)

AST levels were assessed calorimetrically using commercially available diagnostic kits, a widely accepted method known for its precision and reliability in clinical settings. Firstly, these tubes were meticulously labeled as blank, sample and standard. Each of these tubes was filled with 500 µl of color reagents, essential components for the enzymatic reaction. To facilitate the optimal reaction conditions required for accurate AST measurement a 0.4N NaOH solution was added in a sample tube. Subsequently, 200 µl of serum sample was added to all three tubes ensuring uniformity across the analysis. The values obtained from both sample and standard were recorded properly.

3.4.1.3. Alkaline Phosphatase (ALP)

Calorimetry, a technique widely employed in commercial clinical diagnostic kits was utilized to assess ALP (alkaline phosphatase) levels. Initially, three tubes were taken and appropriately marked: one for the blank, one for the standard and one for the sample. Afterwards, 500 µl of chromogenic reagent was added to each tube. This reagent is important because it helps the enzymatic reaction needed for ALP measurement. For the purpose of optimizing the reaction conditions a precise volume of 1 ml of a 0.4N NaOH solution was put into the sample tube. Subsequently, 200 µl of serum sample in each tube was added, ensuring uniformity in the experimental setup. Absorbance values of both the standard and the sample were meticulously noted.

3.4.2. Serum lipid profile

The serum profile consists of measurements of cholesterol, HDL, LDL and triglycerides in rats and was determined using the Merck Commercial Kits technique following the specified methodology of NHANES (2000).

3.4.2.1. Total Cholesterol (TC)

The determination was conducted using the Merck kit method. A 20µL sample and standard solution are mixed with 2ml of working reagent. The test tubes are then vortexed and incubated

for 5 minutes at a temperature of 37 degrees Celsius by placing them in a water bath.

3.4.2.2. Triglyceride (TG)

Merck's commercial kits for triglyceride measurement employs an enzymatic- colorimetric method which is a reliable and widely accepted technique in clinical diagnostics. Briefly, reagent tubes labeled as sample, blank (containing all reagents except the sample) and standard solutions with known concentrations of triglycerides. Appropriate volume of serum/plasma samples, standards and blanks into the wells of a microplate or cuvette was added. Then reagent mixture to each well or cuvette was introduced. The mixture sample was subjected to incubation at 37 degrees for 10-15 minutes. During that time, the triglycerides were hydrolyzed to glycerol and phosphorylated to produce hydrogen peroxide and measured at a 500 nm wavelength using a spectrophotometer. The concentration of triglyceride in the sample was obtained by comparing the absorbance of different samples with standards. The absorbance was proportional to the concentration of triglycerides.

3.4.2.3. High-Density Lipoprotein (HDL)

A volume of 200 µl of plasma was collected in a test tube with 500µl of a diluted reagent.

The ingredients were thoroughly blended and left for 10 minutes. The samples were subjected to centrifugation at a speed of 4000 rev/min for 10-11 minutes' duration. Supernatant volume of 70 µl was taken in a separate test tube from the centrifuged working solution stated before. A volume of 2 ml of cholesterol reagent was added. The absorbance of both known standard solutions and unknown samples was measured at a 505 nm wavelength. Its determination was performed using a commercial diagnostic kit from Merck.

3.4.2.4. Low-Density Lipoprotein (LDL)

Serum LDL level was measured by Friedwald equation according to (Dintshe *et al.,* 2022) as mentioned below:

LDL (mg/dl) = Total Cholesterol - HDL - TG/5

3.4.2.5. Very Low-Density Lipoprotein (VLDL)

VLDL-C is not directly measured but is often estimated as TG divided by a factor of 5.

3.5. Statistical analysis

Statistical results through the application of factorial design using Statistix 8.1 was applied on the final data obtained from this study. The statistical test used to analyze the variance was Two-way ANOVA. The criterion was set at a p-value of less than 0.05 for determining significant results. Completely randomized design test was used to compare mean values of replicated data shown as the average standard deviation (SD) of all parameters.

3.6. Results

3.6.1. Effect of treatments on weight (g) of rats

Table 3.3 shows the average body weight (g) of rats at day 0,14 and 28 of the feeding trial. The growth in body weight was progressive on all treatment groups over the time. At day 0, the starting body weights were between 120.0±4.8 (T0) to 136.5±4.1 (T4). The weights changed, and after 14 days, they were between 128.0±2.6g (T1) to 142.5±2.8 g (T4). And the trend persisted till the end of the day 28, when the final weights ranged between 130.0±2.6g (T1) and 150.0±3.0g (T0). The largest mean body weight after the end of the experiment was recorded by the control group (T0: 150.0±3.0g), then T4 (149.2±3.0g), and T1 with the lowest mean weight (130.0±2.6g). The mean weights at all the study periods were also significantly different ($p < 0.05$) as the minimum was at T1 (127.7±2.4g) and the maximum was at T4 (142.7±6.4g). The statistical tests showed a substantial difference in treatments over the varying time points and these differences were indicated by different superscript (a-g). Altogether, the largest and consistent change of body weight had T4 with the most significant impact of the intervention. T1 by contrast had the lowest increment and this would imply that it has a relatively low impact on growth performance. All means of measurements at day 28 were significantly greater than that of

day 0 (142.5±8.1g vs 128.4±6.3 g), which confirmed the overall trend of weight increase throughout the course of experiment.

Table 3.2 Analysis of Variance for Weight (g) in rat

Source	DF	SS	MS
Days	2	1501.68	750.842**
Treatment	4	1070.10	267.526**
Days*Treatment	8	531.28	66.410**
Error	30	368.31	12.277
Total	44	3471.38	

Table 3.3 Mean Comparison of Weight (g) of rats

Treatment	0	14	28	Mean
T0	120.0±4.8^{g}	135±2.7^{bcdef}	150.0±3.0^{a}	135.0±15^{b}
T1	125.1±5.0^{fg}	128.0±2.6^{efg}	130.0±2.6^{defg}	127.7±2.4^{c}
T2	128.1±5.1^{efg}	134.0±2.7^{bcdef}	140.5±2.8^{abcd}	134.2±6.2^{b}
T3	132.3±4.0^{cdef}	137.2±2.7^{bcde}	143±2.9^{ab}	137.5±5.4^{b}
T4	136.5±4.1^{bcde}	142.5±2.8a^{bc}	149.2±3.0^{a}	142.7±6.4^{a}
Mean	128.4±6.3^{c}	135.34±5.2^{b}	142.5±8.1^{a}	

3.6.2. Effect of treatments on Hemoglobin (Hb) (g/dl) level in rats

Table 3.5 provides the summary of levels of hemoglobin in experimental rats under the various types of treatments and time points (0, 14 and 28 days). The median values of Hb were comparable (14.1 g/dL) in all groups of treatment at the baseline (day 0), which are not different. By the 14th day, T1 (12.1±0.87 g/dL), T2 (12.9±0.17 g/dL), T3 (13.1±0.82 g/dL) and T4 (13.3±0.72 g/dL) had shown a significant dropping effect, T0 (14.4±0.12 g/dL) remained rather stable. This was in T1 that the greatest decrease was observed. The same trend was observed on day 28 with the lowest Hb level of T1 (11.7 mg/dL) and the highest level of T0 (14.5 g/dL) close to its initial level. Across the specified period the averages of hemoglobin were found as the highest in T0 (14.3±0.20 g/ dL) and lowest in T1 (12.6±1.28 g/ dL). With these findings, it is possible to indicate that the treatment administered in T1 stabilized Hb levels, as opposed to the other treatments especially T2 that had negative effects on the levels of hemoglobin over time.

Table 3.4 Analysis of Variance for Hemoglobin (Hb) (g/dl) in rats

Source	DF	SS	MS
Days	2	7.8520	3.92600**
Treatment	4	13.3520	3.33800**
Days*Treatment	8	7.2280	0.90350**
Error	30	0.3000	0.01000
Total	44	28.7320	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.5 Mean Comparison of Hemoglobin (Hb) (g/dl) of rats

Treatment	0	14	28	Mean
T0	14.1±0.93^{b}	14.4±0.12^{ab}	14.5±0.23^{a}	14.3±0.20^{a}
T1	14.1±0.78^{b}	12.1±0.87^{f}	11.7±0.34^{g}	12.6±1.28^{d}
T2	14.1±0.56^{b}	12.9±0.17^{e}	13.2±0.56^{de}	13.4±0.62^{c}
T3	14.1±0.33^{b}	13.1±0.82^{de}	13.4±0.61^{cd}	13.5±0.51^{bc}
T4	14.1±0.45^{b}	13.3±0.72^{cd}	13.6±0.21^{c}	13.7±0.40^{b}
Mean	14.1±0.23^{a}	13.16±0.82^{c}	13.3±1.01^{b}	

3.6.3. Effect of treatment on HCT

The table 3.7 shows that mean hematocrit (HCT) values of the rats grouped under various treatment followed in 0, 14, and 28 days of study. Values of HCT were between 36.3±2.5% to38.2±1.8%, which means that there were no significant differences among the groups ($p < 0.05$)and individuals at the baseline (day 0), indicating initial physiological homogeneity. The negative control group (T0) experienced constant value of HCT during the experiment, and the value also slightly decreased the initial one at day 0 (38.0±1.3%) at day 28 (39.3±1.5%), leading to an overall mean of 37.9±1.4 %. As with the positive control group (T1) that was fed a high-cholesterol diet with no supplementation, there was a lesser decrease in HCT values over period of time, with the lowest reading of 35.7±2.3 at day 14 and an overall mean of 36.7±0.8, which is perhaps a sign of the development of mild anemia or even red blood cell volume change, since the hypercholesterolemia occurred. There were minor elevated or unchanged levels of HCT in supplemented groups. The total mean of Group T2 (chia) was 37.5±0.6% and that of Group T3 (basil) 38.1±0.8%. This has a mild nature of hematinic or erythropoietic effect of the treatment. Group T4 (chia plus basil) also showed a trend of improvement, where the HCT elevated to 38.3±2.9 % at day 28 of the study against 36.3±2.5% at day 0 to bring up an overall Mean of 37.4±1.0 %. Upon averaging with all treatments, there was significant decrease in HCT values at day 14 (36.8±0.7%; Day 28 38.2±0.9%) indicating a general trend of hematological normalization. This data indicates that chia and basil seed supplement might be effective in supporting the healthy levels of hematocrit in cases of hypercholesterolemia.

Table 3.6 Analysis of Variance for HCT in rats

Source	DF	SS	MS
Days	2	13.602	6.80089
Treatment	4	10.399	2.59967
Days*Treatment	8	16.403	2.05033
Error	30	110.060	3.66867
Total	44	150.463	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.7 Mean Comparison of HCT of rats

Treatment	0	14	28	Mean
T0	38.0±1.3^{b}	36.4±2.7^{f}	39.3±1.5^{a}	37.9±1.4^{b}
T1	36.9±2.4^{f}	35.7±2.3^{f}	37.5±2.0^{c}	36.7±0.8^{d}
T2	38.2±1.6^{b}	37.4±2.1^{de}	37.0±1.6^{cd}	37.5±0.6^{b}
T3	38.2±1.8^{b}	37.2±1.5^{de}	38.9±1.5^{b}	38.1±0.8^{a}
T4	36.3±2.5^{f}	37.5±1.4^{cd}	38.3±1.9^{b}	37.4±1.0^{bc}
Mean	37.5±0.9^{b}	36.8±0.7^{c}	38.2±0.9^{a}	

3.6.4. Effect of treatment on RBCs (106/µL)

Table 3.9 represents the average of the red blood cells (RBC) count of the rats in different treatment groups in the 28 days of the experiment. At the first visit (day 0), RBC values were 7.6±2.2 to 8.3±1.9 in the four groups with the general trend showing an increase in RBC counts in groups with chia or basil powder supplementation or combination in supplementation. The positive control group (T1) that was fed on highcholesteroldiet without any dietary intervention had the lowest RBC count at baseline and throughout the study period with mean of 7.7±0.8 on the first day through day 28(7.6±2.2; 7.7±2.1; 7.7±2.1; 7.8±1.8 and 7.8±1.8). This minute rise was in a small series and may be due to inhibited erythropoiesis, or to an absence of stimulating hematinic, with hypercholesterolemic stress. On the contrary, the treatment groups (chia (T2); basil (T3); and their combination (T4 and T5) showed a steady and progressive increase in the RBC count in the 28 days. The T5 group (the combination of chia and basil at the highest dose) exhibited the strongest effect whereby values improved in the combo at day 0 (8.3±1.9) and at day 28 (8.5±1.4) and mean of 8.4±0.08) indicating improvement in erythropoiesis and red blood cell stability. In the same way, there was the strong upward trend of T4, and the final number was 8.36±1.2 with 8.3±0.06 mean. Good changes were also observed in T3 and T2 with overall means of 8.1±0.07 and 8.0±0.10, respectively. The overall trend of RBC count of the average across groups increased progressively with time, beginning with RBC counts of 8.0±0.30 at the baseline point in the first spot to 8.2±0.28 in spot 28, which demonstrates an overall positive trend in the hematological indices, especially in the supplemented groups.

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indices, especially in the supplemented groups.

Table 3.14 Analysis of Variance for HDL (mg/dl) in rats

Source	DF	SS	MS
Days	2	455.34	227.670**
Treatment	4	603.73	150.933**
Days*Treatment	8	335.44	41.930**
Error	30	90.77	3.026
Total	44	1485.29	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.15 Mean Comparison of HDL (mg/dl) of rats

Treatment	0	14	28	Mean
T0	52.4±2.3ab	53.13±1.6a	53.2±1.5a	52.9±0.4a
T1	51.5±2.0ab	39.5±1.1ef	35.2±1.0f	42.1±8.4d
T2	50.4±2.1ab	42.6±0.8cde	47.8±1.4bc	46.9±3.97c
T3	51.2±3.3ab	41.2±1.6de	44.5±1.3cde	45.6±5.07c
T4	53.4±2.2a	44.8±1.3cd	50.5±1.0ab	49.6±4.36b
Mean	51.8±1.15a	44.24±5.33c	46.2±6.96b	

3.6.8. Effect of treatments on VLDL in rats

The trends of the variables of Table 3.17 of levels of very-low-density lipoprotein (VLDL) by different treatment groups during the period of 28 days can be seen in the data presented. The experiment started with the comparable value of VLDL in all the groups (day 0), with no significant difference ($p > 0.05$), which proved that the establishment of the baseline was uniform. Within 14 days, the hyperlipidemic group (T1) had increased greatly in VLDL at 42.5 mg/dl, but on the 28th day had reached the peak at 47.8mg/dl. This led to the maximum in the total VLDL mean (37.8±13.02 mg/dl) demonstrating the ability to induce hyperlipidemia. Conversely, more constrained VLDL response was revealed in the treatment groups (T2, T3, T4). Importantly, T4 group demonstrated the maximal decrease in VLDL at the end of the study, it reduced to 28.4 g/dl at the start of the day 28 relative to 36.9 at one of the days 14. The general average of T4 (28.3±8.58 mg/dl) was considerably below T1 ($p < 0.05$) which demonstrates the possible success of the dietary or therapeutic measure applied in this cohort. The mean values of VLDL in all these treatments drastically increased on the 14th day (36.32±8.03 mg/dl) as compared to the initial values on the 0th day (21.5±1.45 mg/dl) and then recorded slight reduction on the 28th day (32.8±9.43 mg/dl). This trend implies that despite causing the increased VLDL level at the start of the induction type, diet due to the selected interventions, and especially T4, succeeded in diminishing VLDL concentration, thus implying that lipid metabolism and cardiovascular risks were positively influenced by the interventions.

Table 3.16 Analysis of Variance for VLDL (mg/dl) in rats

Source	DF	SS	MS
Days	2	1795.27	897.636**
Treatment	4	1113.01	278.253**
Days*Treatment	8	755.57	94.447**
Error	30	55.71	1.857
Total	44	3719.57	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.17 Mean Comparison of VLDL (mg/dl) of rat

Treatment	0	14	28	Mean
T0	22.6±1.6g	22.4±0.6g	22.3±0.6g	22.4±0.13d
T1	23.1±1.9g	42.5±1.2b	47.8±1.4a	37.8±13.02a
T2	21.9±1.6g	39.6±1.5bc	31.8±0.9ef	31.1±8.85b
T3	20.2±1.8g	40.2±1.2bc	33.6±1.3de	31.3±10.17b
T4	19.7±1.6g	36.9±1.4cd	28.4±1.1f	28.3±8.58c
Mean	21.5±1.45c	36.32±8.03a	32.8±9.43b	

3.6.9. Effect of treatments on Total cholesterol in rats

The total cholesterol levels in rats varied significantly in response to treatment and time points. Statistical homogeneity at baseline was demonstrated since all groups had statistically identical cholesterol levels (ranging between 84.5 and 88.3 mg/dl at day 0). There were vast improvements that were registered on day 14 and 28, especially in T1 with a peak of 165.3±3.3 mg/dl, the highest point recorded on cholesterol during the whole trial. This category also had the highest total mean cholesterol level (134.3±41.5 mg/dl) indicating a definite state of hypercholesterolemia ($p < 0.05$). Treatment groups T2, T3, and T4 were on the contrary enjoying different levels of cholesterol-reducing effect. The greatest improvement was as manifested by T4 whose value returned to only slightly elevated, 105.17 mg/dl at day 14 and then back to normal 95.51 mg/dl at day 28. The average of T4 was 95.0±10.3 mg/dl, which is considerably lower than T1 and indicates the beneficial impact of the administered intervention in decreasing the levels of the lipids. The mean cholesterol concentration across the set of groups significantly rise between day 0 (86.3±1.5 mg/dl) and day 14 (119.94±24.1 mg/dl) displaying some sort of partial recovery mode and minimally dropping by day 28 (115.4±29.7 mg/dl). This pattern indicates a successful increase in cholesterol levels with dietary induction, and the reduction of cholesterol levels, which occurred in specific groups of treatment has indicated the possible therapeutic potential of the bioactive compounds used, in particular those provided in T4.

Table 3.18 Analysis of Variance for Total Cholesterol level (mg/dl) in rats

Source	DF	SS	MS
Days	2	9986.4	4993.21**
Treatment	4	11467.2	2866.81**
Days*Treatment	8	6136.8	767.10**
Error	30	157.1	5.24
Total	44	27747.5	

Table 3.19 Mean Comparison of Total Cholesterol level (mg/dl) of rats

Treatment	0	14	28	Mean
T0	85.2±1.7g	88.03±1.7g	90.2±1.8fg	87.8±2.5d
T1	87.1±2.6g	150.6±3.0b	165.3±3.3a	134.3±41.5a
T2	86.5±2.5g	130.6±2.6c	115.47±2.3d	110.9±22.4b
T3	88.3±1.7g	125.3±2.5c	110.75±2.2de	108.1±18.6b
T4	84.5±1.6g	105.17±2.1e	95.51±1.9f	95.0±10.3c
Mean	86.3±1.5c	119.94±24.1a	115.4±29.7b	

3.6.10. Effect of treatments on ALT in rats

Table 3.21 shows the mean ALT levels (u/L) of rats in the various treatment groups in day 0, 14 and 28. There was no statistical significant difference between the baseline (day 0) ALT levels of all the groups ($p > 0.05$) and the values ranged between 29.4±1.5 to 32.1±1.7 u/L, which is evidence that there was no significant difference in the way any of the groups performed before treatment. Nevertheless, the big differences ($p < 0.05$) were recorded after 14 and 28 days in the treatment groups. The T0 control group did not exhibit damage in its ALT levels with minimal change (32.1±0.6 u/L mean) over the period of the study, which affirmed the lack of hepatic stress or damage. The positive control group (T1), on the contrary, that was exposed to hypercholesterolemic conditions in the absence of any supplementation demonstrated that significant increase in the ALT levels up to 72.5±2.9 u/l on the 28th days and overall average of 57.1±23.0 u/l, which proves the presence of liver damage or stress. The T2, T3 and T4 groups, treated with chia, alone or in combination of basil dietary supplements, exhibited significant dropping in the ALT levels relative to T1. In particular, group T4 demonstrated the most dramatic decrease, with ALT level of 42.2±1.6 u/L at the time point of 28 days and the mean value of 42.8±11.6 u/L. Likewise, the hepatoprotective effect of T3 and T2 was illustrated in the pieces of 47.6 ±14.3 and 45.9±15.3 u/L, respectively. In sum, the average ALT result of all the treatments on basal state (31.0±13.5 u/L) was lowest ,while on day 14 (55.06±3.5 u/L)and on day 28 (49.3± 4.8 u/L) it was highest.

Table 3.20 Analysis of Variance for Alanine Transaminase (ALT) (u/L) in rats

Source	DF	SS	MS
Days	2	4735.40	2367.70**
Treatment	4	2928.52	732.13**
Days*Treatment	8	1911.97	239.00**
Error	30	106.72	3.56
Total	44	9682.61	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.21 Mean Comparison of Alanine Transaminase (ALT) (u/L) of rats

Treatment	0	14	28	Mean
T0	$31.4±2.2^{f}$	$32.5±1.3^{f}$	$32.3±1.2^{f}$	$32.1±0.6^{d}$
T1	$30.5±1.2^{f}$	$68.2±2.7^{a}$	$72.5±2.9^{a}$	$57.1±23.0^{a}$
T2	$29.4±1.5^{f}$	$59.6±2.3^{b}$	$48.8±1.9^{d}$	$45.9±15.3^{b}$
T3	$32.1±1.7^{f}$	$60.3±1.2^{b}$	$50.5±2.0^{cd}$	$47.6±14.3^{b}$
T4	$31.5±2.1^{f}$	$54.7±1.6^{bc}$	$42.2±1.6^{e}$	$42.8±11.6^{c}$
Mean	$31.0±1.04^{c}$	$55.06±13.5^{a}$	$49.3±14.8^{b}$	

3.6.11. Effect of treatments on AST in rats

Table 3.23 shows the mean values of Aspartate Aminotransferase (AST) activityof the rat measured at day 0, 14 and 28. By baseline (day 0) the AST values were notstatistically different among all study groups ($p > 0.05$), with values of35.7±1.3 µ/L, showing that there was no pre-existing hepatic disparity among the groups. On the 14th day and 28th day, marked ($p < 0.05$) increase in the levels of AST was recorded in the positive control (T1) group, at 91.5±2.8 to 70.5±32.5 (overall mean). This brusque rise demonstrates that rats fed a hypercholesterolemic diet without treatment have the possibility of liver damage or metabolic distress. On the contrary, group 2 (T2), 3 (T3), and 4 (T4) treatment groups with chia, basil, and combination of chia and basil, showed

significantly low values of AST compared to that of T1. The greatest hepatoprotective effect was observed in the T4 group with AST levels of 58.2±1.6. At the end of day 28; the levels were significantly higher and the mean of this group was 53.42±18.1 indicating that both individual and combined supplementation using seeds were effective to relieve the stress in the liver caused by hypercholesterolemia. The control group (T0) showed a stable and low level of AST at each time point and a mean value of 35.8±0.4 u/L, which reinforced the fact that there was no hepatic damage observed in the untreated rats. The levels of AST were the highest on the 14th day of treatment (67.8±19.2 µ/L), slightly decreased on the 28th day (63.4±19.8 µ/L), and were the lowest at baseline (33.8±1.2 µ/L) when averaged across all treatments.

Table 3.22 Analysis of Variance for Aspartate Amino Transferase (µ/L) in rats

Source	DF	SS	MS
Days	2	10276.2	5138.09
Treatment	4	5637.8	1409.46
Days*Treatment	8	3555.4	444.42
Error	30	107.8	3.59
Total	44	19577.2	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.23 Mean Comparison of Aspartate Amino Transferase (µ/L) of rats

Treatment	0	14	28	Mean
T0	$35.7±1.3^{f}$	$35.5±1.2^{f}$	$36.2±1.1^{f}$	$35.8±0.4^{d}$
T1	$33.1±1.5^{f}$	$86.9±3.0^{a}$	$91.5±2.8^{a}$	$70.5±32.5^{a}$
T2	$32.5±1.4^{f}$	$75.2±2.2^{b}$	$64.4±1.9^{d}$	$57.4±22.2^{b}$
T3	$34.3±2.0^{f}$	$72.8±1.8^{bc}$	$66.9±2.0^{d}$	$58.0±20.7_{b}$
T4	$33.4±1.6^{f}$	$68.6±2.1^{cd}$	$58.2±1.6^{e}$	$53.4±18.1^{c}$
Mean	$33.8±1.2^{c}$	$67.8±19.2^{a}$	$63.4±19.8^{b}$	

3.6.12. Effect of treatments on ALP in rats

The data analysis represents the mean values of Alkaline Phosphatase (ALP) activity in rats during the period of study represented in Table 3.25. A significant increase in the ALP was observed on day 0 in the hyper cholesterolemic groups compared to T0 (Day 0), where T1 had the highest initial value (68.2±2.7 u/L), whereas T0 recorded a having a basal value of 32.4±1.3u/L ($p < 0.05$). Essentially, in the T0 group, the results remained stable during the experiment, the overall mean of 32.2±0.2 u/L indicated the presence of normal hepatic functions. There was a marked liver stress or biliary obstruction as evidenced by the significantly high level of ALP on control (T1) which were not supplemented throughout the period of study and its average was 72±3.6 µ/L. Conversely, T2 [treatment with chia], T3 [treatment with basil] and T4 [treatment with the combination of chia and basil] had shown a great decrease in the level of ALP with elapsed time as compared to T1. Under the supplemented groups, T4 (combined chia and basil) was the one with the most striking reduction in the level of ALP, 45.6±8.1 µ/L mean, followed by T2 (50.6±8.2 µ/L mean) and T3 (52±7.6 µ/L mean). These decreases allude to a hepatoprotective impact and liver performance in rats which got the seed- based interventions. The trend in all the treatments was that maximum average ALP was on day 0 (55.04±13.5), which was later decreased as the days progressed to 49.26±14.8 on day 14 and 47.16±16.5 on day 28. These results substantiate the conclusion that dietary supplementation with chia and basil seed powder have the capability of moderating the rise of ALP precipitated by hypercholesterolemia, thus signifying their probable usage in hepatic health.

Table 3.24 Analysis of Variance for Alkaline Phosphatase (ALP) (µ/L) in rats

Source	DF	SS	MS
Days	2	499.56	249.78**
Treatment	4	7413.75	1853.44**
Days*Treatment	8	725.72	90.71**
Error	30	120.86	4.03
Total	44	8759.89	

*=significant ($p < 0.05$); **= highly significant ($p < 0.01$); NS= non-significant

Table 3.25 Mean Comparison of Alkaline Phosphatase (ALP) (µ/L) of rats

Treatment	0	14	28	Mean
T0	32.4±1.3^{h}	32.3±1.2^{h}	32.1±1.3^{h}	32.2±0.2^{d}
T1	68.2±2.7^{b}	72.5±2.9^{ab}	75.4±3.2^{a}	72±3.6^{a}
T2	59.6±2.3^{c}	48.8±1.9def	43.6±1.8f^{g}	50.6±8.2^{b}
T3	60.3±1.2^{c}	50.5±2de	45.2±2.1efg	52±7.6^{b}
T4	54.7±1.6^{cd}	42.2±1.6^{g}	39.5±1.6g	45.6±8.1^{c}
Mean	55.04±13.5^{a}	49.26±14.8^{b}	47.16±16.5^{c}	

3.6.13. Effect of treatments on Platelets in rats

Table 3.27 summarizes the mean counts of platelets at day 0, 14 and 28 in therats. The platelets at the baseline (day 0) were also similarly distributed in all the treatment groups with values between 351.5±10.7 and 353.4±10.9, and therefore there was no significant difference in the platelets among the groups ($p > 0.05$). Nevertheless, significant differences in the number of platelets were found in the following days according to the diet treatment. The negative control group (T0) showed a relatively stable platelet count that dropped slightly on day 14 (288.5±13.2) and regained subsequently on day 28 (357.5±11.1) to give an overall value of 332.9±38.5. Hypercholesterolemic group which did not receive any supplementation (T1) showed a steady decrease in the number of platelets as the time progressed with day 28 having a significantly reduced number (280.3±11.8) and an overall aver-age of 306.8±38.9 which indicates that a diet rich in cholesterol can disrupt red platelet production or cause them to be consumed. In their turn, all the groups treated with chia (T2), basil (T3), and chia and basil combination (T4) tended to normalize and even increase the number of platelets on day 28. It is noteworthy that group T4 had the best platelet count value at the 28th day (339.4±11.8) and the overall mean (340.5±10.5), which indicates high restoration or protection activity. Equally, T3 and T2 obtained the mean values of 331.0±18.9 and 328.4±22.3, respectively, indicating the positive effects of these seed powders to enhance the healthy level of platelet. Platelet counts were lowest on day 14 (306.9±18.3) and yet there was a significant increment on day 28 (324.6±28.6) of the study implying recovery with regard to dietary manipulation.

Table 3.27 Mean Comparison of Platelets (103/µL) of rats

Treatment	0	14	28	Mean
T0	352.6±10.3^{ab}	288.5±13.2^{ef}	357.5±11.1^{a}	332.9±38.5^{a}
T1	351.5±10.7a^{bc}	288.4±12.8^{ef}	280.3±11.8^{f}	306.8±38.9^{b}
T2	353.4±10.9^{ab}	310.4±11.8d^{ef}	321.5±12.2^{bcde}	328.4±22.3^{a}
T3	352.4±11.1^{ab}	316.4±12.9^{cde}	324.3±13.1^{abcd}	331.0±18.9^{a}
T4	351.5±11.2^{abc}	330.6±12.4^{abcd}	339.4±11.8^{abcd}	340.5±10.5^{a}
Mean	352.3±0.8^{ab}	306.9±18.3^{c}	324.6±28.6^{b}	

3.6.14. Effect of treatments on Triglycerides in rats

Table 3.29 contains the mean values of triglycerides (mg/dL) of rats under various treatments and at various timepoints. The level of triglyceride at baseline (day 0) was similar in all groups with a range of 91.1±1.6 to 110.6±1.6 mg/dL. The negative control group (T0) had constant and low levels of triglycerides over the period of the study, with overall average levels to 91.6±0.5 mg/dL, which proved that there was a stable metabolism in the absence of a hypercholesterolemic diet.Conversely, the positive control group (T1), given only a high-cholesterol diet, showed a drastic and significant augmentation in the level of triglycerides, suchas 210.1±2.4 mg/dL on day 28, and a total mean of 168.9±51.9 mg/dL. This drastic increase signifies the hyperlipidemiceffects of the diet and possible dysfunction of metabolism. Treatment group T2 (chia), T3 (basil) and T4 (chia + basil) significantly dropped the triglyceride levels as compared to the positive control. It isworth noting that T4 had the greatest hypolipidemic activity and measured 117.8±1.6 to 122.2±14.8 mg/dL after 28 days and a total mean of 122.2±14.8 mg/dL. The positive outcomes were also observed with T2 and T3 at which the means of triglycerides were 125.6±18.5 and 132.5±23.5 mg/dL, respectively. Such observations indicate that chia and basil seed supplementation, particularly a combination thereof, has a potential tosignificantly relieve the risk of triglyceride induction related to hypercholesterolemia. The general trend of the treatments showed that levels of triglyceride showed the peak at the 14th day (143.82±34.2 mg/dL) and has a slight decrease after 28 days (134.4±44.7 mg/dL), which could show that dietary interventions may take time to respond. These findings substantiate the lipid-lowering effects of chia and basil seeds and, especially, their combination in the case of hypercholesterolemia and the high level of triglycerides

Table 3.28 Analysis of Variance for Triglycerides (mg/dl) in rats

Source	DF	SS	MS
Days	2	11421.1	5710.57**
Treatment	4	27465.9	6866.48**
Days*Treatment	8	11375.5	1421.93**
Error	30	102.9	3.43
Total	44	50365.4	

Table 3.29 Mean Comparison of Triglycerides (mg/dl) of rats

Treatment	0	14	28	Mean
T0	91.1±1.6^{i}	91.8±1.5i	92.0±1.6^{i}	91.6±0.5^{e}
T1	110.6±1.6^{h}	185.9±1.7^{b}	210.1±2.4^{a}	168.9±51.9^{a}
T2	109.5±2.0^{h}	145.7±2.2$_{d}$	121.6±1.9^{g}	125.6±18.5^{c}
T3	110.1±1.7^{h}	157.0±1.8^{c}	130.4±1.6^{f}	132.5±23.5^{b}
T4	110.2±2.6^{h}	138.7±1.7^{e}	117.8±1.6^{g}	122.2±14.8^{d}
Mean	106.3±8.5^{c}	143.82±34.2^{a}	134.4±44.7^{b}	

Discussion

The proximate and biochemical analysis of chia (Salvia hispanica) and basil (Ocimum basilicum) seeds demonstrated their strong nutritional and functional potential for food and nutraceutical applications. Both seeds showed acceptable moisture levels below 10%, indicating good shelf stability and reduced microbial susceptibility. Chia seeds exhibited higher crude fat (30.45 ± 0.35%) and dietary fiber content (34.65 ± 0.28%) compared to basil seeds, reflecting their richness in omega-3 fatty acids and functional fiber components (Mohd Ali et al., 2012; Reyes-Caudillo et al., 2008). In contrast, basil seeds

contained comparatively higher ash content (5.76 ± 0.18%) and total phenolic content (18.31 ± 0.58 mg GAE/g), suggesting superior mineral composition and antioxidant potential (Rababah et al., 2004). Moreover, basil seeds demonstrated stronger DPPH radical scavenging activity (65.89 ± 2.07%) than chia seeds (58.14 ± 1.62%), confirming their enhanced free radical neutralizing capacity. These findings support previous reports emphasizing the functional and therapeutic importance of both seeds in reducing oxidative stress and improving metabolic health (Taga et al., 1984; Ullah et al., 2016).

The biological evaluation conducted on hypercholesterolemic albino rats further highlighted the therapeutic efficacy of chia and basil seed supplementation. Rats receiving high-cholesterol diets without supplementation (T1) exhibited greater body weight gain, disturbed hematological indices, elevated lipid parameters, and impaired liver enzyme profiles. In contrast, supplementation with chia (T2), basil (T3), and especially their combination (T4) significantly improved physiological and biochemical markers ($p < 0.05$). The combination treatment (T4) effectively controlled body weight gain and improved hematological parameters including RBC, WBC, hematocrit, platelet count, and hemoglobin levels, indicating possible erythropoietic and immunomodulatory effects (Aziz et al., 2020; Rafique et al., 2020). Previous studies also reported similar improvements in hematological stability following supplementation with functional plant-based ingredients in hyperlipidemic models (Ali et al., 2019; Sohail et al., 2020).

Significant hypolipidemic effects were observed in the supplemented groups, particularly in T4, where LDL, VLDL, triglycerides, and total cholesterol levels markedly decreased while HDL concentrations improved. The untreated hypercholesterolemic group showed severe dyslipidemia with elevated LDL and triglycerides, whereas combined chia and basil supplementation reduced LDL to 42.67 mg/dL and improved HDL to 50.6 mg/dL by day 28. These outcomes are consistent with earlier studies demonstrating the cholesterol-lowering and cardioprotective properties of chia and basil seeds due to their omega-3 fatty acids, dietary fiber, and antioxidant compounds (Aslam et al., 2019; Ullah et al., 2016; Farooq et al., 2021). Similarly, triglyceride and VLDL reductions observed in treated groups align with findings of Aydin et al. (2018) and Iftikhar et al. (2021), who reported substantial lipid profile improvements after basil and chia supplementation.

Liver function biomarkers including ALT, AST, and ALP were significantly elevated in untreated hypercholesterolemic rats, indicating hepatic stress and possible liver dysfunction induced by excessive cholesterol intake. However, supplementation with chia and basil seeds significantly reduced these enzyme levels, with the combined treatment group showing the greatest hepatoprotective effect. These results are supported by previous investigations reporting that chia and basil possess antioxidant and anti-inflammatory constituents capable of protecting hepatic tissues against lipid-induced oxidative damage (Abdel-Salam et al., 2020; Mohammed et al., 2018; Farag et al., 2021). Overall, the present study confirms that chia and basil seeds, particularly in combination, possess strong nutritional, antioxidant, hypolipidemic, hematoprotective, and hepatoprotective properties. Therefore, their incorporation into functional foods and nutraceutical formulations may provide an effective dietary strategy for the management of hypercholesterolemia and associated metabolic disorders.

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