



# Exploring the Therapeutic Effect of Beetroot-Sweet Basil Pestils on Hyperlipidemia

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**Abstract:** Hyperlipidemia, a major public health challenge, is a leading risk factor for cardiovascular morbidity and mortality. Plant-based dietary interventions are being recognized as effective strategies for its prevention and management. Beetroot (*Beta vulgaris* L.) and sweet basil (*Ocimum basilicum* L.) possess antioxidant and anti-inflammatory properties, making them promising candidates for functional food development. In this study, beetroot-sweet basil pestils were formulated by incorporating sweet basil leaf powder at varying concentrations (0–20%). Proximate analysis indicated significant ( $p < 0.05$ ) increases in protein (1.75-fold), fiber (1.32-fold), and ash content (2.51-fold) compared with the control. Phytochemical evaluation further demonstrated a concentration-dependent enhancement in total phenolic content, total flavonoid content, and antioxidant activity. Among formulations, the 5% sweet basil pestil was identified as optimal and administered to hyperlipidemic rats at doses of 100 and 150 mg/kg body weight/day for 30 days. Serum lipid analysis revealed significant ( $p < 0.05$ ) elevations in high-density lipoprotein levels from 30 to 34.6 mg/dL along with significant reductions in total cholesterol from 221.6 to 206.4 mg/dL, low-density lipoprotein from 164.8 to 156 mg/dL, and triglycerides from 165 to 154.8 mg/dL in treatment groups compared to the diseased control group. These findings highlight the potential of beetroot-sweet basil pestil as a novel functional food with therapeutic efficacy against hyperlipidemia and associated cardiovascular risks.

**Keywords:** hyperlipidemia; beetroot; sweet basil; pestil; lipid profile

## 1. Introduction

Hyperlipidemia, a lipid metabolism disorder, involves an abnormal upsurge in serum lipid levels, marked by increased plasma concentration of total cholesterol, LDL cholesterol and triglycerides and reduced HDL cholesterol. The main risk factors contributing to hyperlipidemia include lifestyle factors such as unhealthy diets, physical inactivity, genetics, underlying conditions like diabetes, overweight and obesity [1]. The prevalence rates of dyslipidemia ranges from 32.8% to 75.5% among US adults, with hypercholesterolemia affecting up to 12% of the population [2].

Hyperlipidemia is intricately linked to cardiovascular diseases and increased mortality risk. In Pakistan, prevalence of hyperlipidemia has surged to 30%, rendering it a pressing public health problem [3].

Plant-based interventions because of their affordability and evidence-based therapeutic effects are in trend for better managing cardiovascular diseases and associated complications. According to World Health Organization, 90% of the population in developing countries use herbal remedies and nutraceuticals to help prevent, control or improve non-communicable diseases such as hyperlipidemia, obesity, hypertension and cardiovascular diseases etc. [4,5]. Vegetables and herbs are rich in bioactive compounds such as phenolic acids, carotenoids, polyphenolic compounds, vitamin C and dietary nitrates that possess strong antioxidant and anti-inflammatory potential for preventing and managing cardiovascular diseases.

Beetroot (*Beta vulgaris* L.), a nutritionally rich vegetable from the Chenopodiaceae family, has gained attention for its high content of minerals, vitamins, flavonoids, and bioactive compounds (nitrates and betalains), which contribute to its antioxidant, anti-inflammatory, and cardio protective effects [6]. These attributes make beetroot a promising component in managing conditions like hyperlipidemia, hypertension, cardiovascular disease and diabetes [7].



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Sweet basil (*Ocimum basilicum* L.), is a herb recognized for its therapeutic properties, including anti-inflammatory and antioxidant activities. It has long been used to support cardiovascular health, manage blood pressure, and improve metabolic functions [8]. The use of sweet basil, an aromatic herb can improve the functional profile and novelty of products as well [9].

Despite these benefits, beetroot and sweet basil belongs to relatively unpopular foods consumed rarely as a part of regular diet. Moreover, high moisture content and perishability cause substantial post-harvest losses, with seasonal availability also limiting the consistent consumption. In Pakistan, 21.4% of daily fruits and vegetables are wasted due to limited preservation methods [10]. There is a need for such an approach that help preserve the fruits and vegetables and overcome the challenges of seasonal availability. Functional food products such as fruit and vegetable leathers have chewy texture in the form of strips or flexible sheets, dried nutrient-dense snack, preserves nutrients and enhances the shelf life of seasonal produce, offering a convenient means to consume essential nutrients in off season [11].

The current research involved the production of pestil, a traditional dried fruit product, by concentrating fruit pulp or juice and dehydrating it to a thin, flat layer. The beetroot-sweet basil pestil, a traditional fruit leather formulation, was formulated in different concentrations of sweet basil leaf powder, and their therapeutic properties were evaluated on hyperlipidemic rats.

## 2. Materials and Methods

### 2.1. Procurement of Raw Materials and Reagents

Raw materials were purchased from the local market. All chemicals and reagents required for the present study were of analytical grade and purchased from local market in Faisalabad.

### 2.2. Preparation of Raw Materials

Fresh beetroot was rinsed with water to remove soil and dirt residues. The vegetal parts different from roots were removed and the root was peeled. Afterwards, beetroot puree was prepared by using an electric blender and stored for further use (Vitamix 5200, VC, Vitamix, Cleveland, OH, USA). The spoilt and damaged parts of the sweet basil leaves were separated from the fresh leaves and washed with tap water to remove the contaminants. The leaves were placed in sunlight until complete drying followed by grinding and sieving to obtain sweet basil powder of uniform particle size [12]. Finally, the powder was packed in polythene bags and stored at room temperature for further study.

### 2.3. Preparation of Beetroot-Sweet Basil Pestil

Beetroot-sweet basil pestils were prepared by substituting beetroot puree with various concentrations of sweet basil leaves powder as mentioned in Table 1 according to the protocol described by [13].

**Table 1.** Treatment plan of beetroot-sweet basil pestils.

| Treatments     | Beetroot Puree (%) | Sweet Basil Leaves Powder (%) |
|----------------|--------------------|-------------------------------|
| T <sub>0</sub> | 100                | 0                             |
| T <sub>1</sub> | 95                 | 5                             |
| T <sub>2</sub> | 90                 | 10                            |
| T <sub>3</sub> | 85                 | 15                            |
| T <sub>4</sub> | 80                 | 20                            |

### 2.4. Analysis of Beetroot-Sweet Basil Pestils

#### 2.4.1. Proximate Analysis

The proximate composition (moisture, crude fat, crude protein, crude fiber and ash) of beetroot-sweet basil pestils was determined following the method of [14]. The moisture content was determined by drying the samples in hot air oven at 105 °C. The samples were weighed before and after oven drying. The crude fat content was measured using Soxhlet apparatus with n-hexane as the solvent, over six extraction cycles. The crude protein content of pestils was determined using the Kjeldahl method using the Kjeltex machine (Technich GmbH, Behr Labor, Germany) by estimating nitrogen content. The nitrogen content was multiplied by conversion factor (6.25) to calculate crude protein. For determination of crude fiber, the pestil samples were treated with sulfuric acid and sodium hydroxide to remove acid and alkali soluble substances using Labconco Fibertech equipment. The acid

and alkali treated samples were charred in a muffle furnace (MF-1/02, PCSIR, Pakistan) at  $600 \pm 2$  °C to determine the ash content of samples.

#### 2.4.2. Mineral Analysis

The concentration of sodium and potassium in beetroot-sweet basil pestils was assessed using an atomic absorption spectrophotometer (Thermo Scientific ICAP 6000, Thermo Scientific, Cambridge, UK). 5 mL of perchloric acid (HClO<sub>4</sub>) and 10 mL of nitric acid (HNO<sub>3</sub>) were mixed with 0.5 g of the dried sample in a digestion flask. On a hot plate, the mixture was heated until it became transparent. Then digested sample was filtered and diluted with distilled water to reach a final volume of 100 mL. The diluted samples were analyzed using spectrophotometer, and the concentration of sodium and potassium was determined by comparing the absorbance values with standard calibration curves prepared for each mineral [15].

#### 2.4.3. Phytochemical Characterization

##### Total Phenolic Content (TPC)

TPC of beetroot pestil was determined by using the Folin-Ciocalteu [16] method with slight modification. 1 g of the sample was dissolved in 10 mL methanol and kept at room temperature for two hours, followed by the filtration using Whatman filter paper No.2. Briefly, 2.5 mL of 7.5% sodium bicarbonate (NaHCO<sub>3</sub>) was added after the addition of extract and Folin-Ciocalteu (FC) reagent 1:1 (v/v) ratio. After that, the mixture was placed at room temperature for half an hour. Using a UV-visible spectrophotometer (Thermo Evolution 220, Thermo Electron Scientific Instrument LLC, Madison, WI, USA), absorbance was measured at 765 nm. The results were expressed as mg GAE/100 g [17].

##### Total Flavonoids Content (TFC)

The aluminium chloride colorimetric method was used to estimate the TFC of beetroot pestil as described by [18] with slight modification. 500 µL of beetroot extract, 2.8 mL of distilled water, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminium chloride, and 1.5 mL of methanol were mixed in a test tube. The mixture was kept at room temperature for 30 min. Then absorbance of solution was measured using a UV-visible spectrophotometer (Hitachi, Tokyo, Japan) at 415 nm. Results are shown as mg CE/100 g.

##### Anti-Oxidative Potential (DPPH)

The antioxidant activity of pestil was calculated by using the DPPH method [19]. 0.025 g of DPPH (1,1-Diphenyl-2-picrylhydrazyl) was dissolved in 1 L of methanol to prepare a solution. Then, 1 mL of the extract was mixed with 3 mL of the DPPH solution, and the solution was left in the dark for 40 min. The absorbance of solution was measured using UV-visible spectrophotometer at wavelength 517 nm. The following formula was used to determine antioxidant activity.

$$\text{DPPH scavenging (\%)} = 1 - \frac{A_f}{A_o} \times 100$$

where,  $A_o$  represents the absorbance of the blank (DPPH solution without the addition of pestil), and  $A_f$  represents the absorbance of the pestil samples.

#### 2.5. Color Analysis

The color of beetroot-sweet basil pestils was analyzed using a HunterLab colorimeter [20]. The L\*, a\* and b\* values were measured at multiple points on each pestil. The L\* value indicates brightness, a\* indicates red-green values, and b\* indicates yellow-blue values. The color difference (“ΔE”), chroma (C\*), and whitening index (WI) were calculated as follows:

$$\Delta E = \sqrt{(L^* - L^*_{std})^2 + (a^* - a^*_{std})^2 + (b^* - b^*_{std})^2}$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

Here, std indicates the control sample.

## 2.6. Sensory Evaluation

The beetroot-sweet basil pestils were analyzed for sensory attributes, including texture, flavor, taste, appearance, and overall acceptability using 9-point hedonic scale. A panel of twenty semi-trained panellists performed the testing of pestil samples. The control sample, without sweet basil, was a reference for comparative scoring. The composition of each sample was concealed from the panellists to minimize potential bias. Panelists were permitted to reassess the reference sample as needed to maintain consistent scoring standards. Additionally, to ensure palate neutrality, panellists rinsed their mouths with water between samples [21]. Based on sensory acceptability and better nutritional profile the best formulation of beetroot-sweet basil pestil was selected for evaluating its therapeutic potential against hyperlipidemia in rat models.

## 2.7. Efficacy Trial

The study was carried out to assess the antihyperlipidemic potential of beetroot-sweet basil pestils. The animal efficacy trial was carried out in National Institute of Food Science and Technology, University of Agriculture, Faisalabad with the approval of the Institutional Biosafety and Bioethics Committee (2041/ORIC), UAF Pakistan. For this purpose, twenty Sprague Dawley rats were procured and housed in the animal room at UAF. All rats were kept in distinct cages, provided with proper ventilation facilities and acclimatized for 7 days while fed on standard rat diet. After acclimatization, the rats were randomly divided into four groups, containing five rats in each group. The period of experimental trial was 30 days. Hyperlipidemia was induced in group G<sub>1</sub>, G<sub>2</sub>, G by administering a high-fat diet for fifteen days prior to the intervention following [22] with slight modification. The intervention period continued for thirty days, during which the control groups were fed on normal rat diet whereas the intervention groups were administered beetroot-sweet basil pestil containing diet (Table 2) to distinguish the effect of feed on serum lipid profile (LDL, HDL, total cholesterol, and TG).

**Table 2.** Experimental groups and dietary interventions for evaluating the anti-hyperlipidemic potential of beetroot-sweet basil pestils in rats.

| Experimental Groups | Treatments                                       | Subjects |
|---------------------|--------------------------------------------------|----------|
| G <sub>0</sub>      | Normal diet (negative control)                   | 5        |
| G <sub>1</sub>      | Normal diet (positive control)                   | 5        |
| G <sub>2</sub>      | Normal diet + beetroot pestil (100 mg/kg bw/day) | 5        |
| G <sub>3</sub>      | Normal diet + beetroot pestil (150 mg/kg bw/day) | 5        |

## 2.8. Serum Lipid Profile

Serum lipid profile including determination of low-density lipoprotein (LDL), total cholesterol, triglyceride along with high-density lipoprotein (mg/dL) was performed at baseline, 15th and 30th day of intervention [23]. The arterial blood samples were collected in simple and Ethylenediamine tetra-acetic acid solution containing tubes using disposable syringes and kept frozen until analyzed.

## 2.9. Statistical Analysis

Each experiment was carried out in triplicates, and results were presented as mean values along with standard deviation. The mean values obtained from product analysis and animal efficacy trial were compared using ANOVA, followed by Tukey's HSD test, with  $p < 0.05$  considered statistically significant using Statistix 8.1 software.

## 3. Results and Discussions

Disease prevention and health promotion strategies are shifting towards plant-based interventions owing to their evidence-based nature, affordability, and accessibility. In this milieu, the current study was conducted to develop and characterize beetroot-sweet basil pestils and evaluation of their antihyperlipidemic potential in rats. The proximate composition, mineral content, antioxidant profile, color and sensory acceptability was evaluated followed by animal trial. Hyperlipidemia was induced and confirmed by analyzing serum lipid profile at baseline followed by measurement at 15th and 30th day for comparison with baseline values. The results and discussion are described below and the investigated parameters are interpreted statistically to draw valid conclusions.

### 3.1. Proximate Composition

The proximate composition of beetroot pestil was determined and results are displayed in Table 3. Results showed that the incorporation of sweet basil leaves powder in beetroot pestil had a significant effect on the proximate composition. The moisture content of beetroot pestils significantly decreased by 4.25% in T<sub>1</sub>, 8.68% in T<sub>2</sub>, 18.01% in T<sub>3</sub>, and 26.54% in T<sub>4</sub> relative to control. This progressive decrease in moisture content may be attributed to the addition of sweet basil leaves powder as it has inherently low moisture as compared to beetroot. Low moisture is desirable as it reduces the susceptibility of microbial spoilage and extends the shelf life [24]. These findings align with [25] where the addition of sweet basil in chicken meatballs decreased the moisture content. Similarly, fat content decreased progressively with increasing sweet basil leaf powder concentration, from T<sub>0</sub> (7.63%) to T<sub>4</sub> (2.05%). This reduction may be attributed to the lower fat content of sweet basil leaves compared to beetroot. These results are correlated with the findings of [26], who stated that the addition of sweet basil decreased the fat content in muffins. Based on crude protein analysis, the crude protein percentages ranges from  $4.26 \pm 0.20$  to  $7.48 \pm 0.16$ . It indicates that the crude protein contents significantly ( $p < 0.05$ ) increased along with the addition of sweet basil powder and this aligns with previously reported literature [27]. The higher the concentration of sweet basil added, the higher the protein content. This increase can be attributed to the higher protein content of sweet basil leaves (3.2 g/100 g) [28] compared to beetroot (1.6 g/100 g) [29], which enriched the overall protein profile of pestils. Similar to crude protein, the crude fiber content showed an increasing trend similar to [27]. The ash percentage also increased from  $1.85 \pm 0.05$  in T<sub>0</sub> to  $4.65 \pm 0.09$  in T<sub>4</sub>. The greater mineral content of sweet basil (*Ocimum basilicum* L.) leaves which are abundant in vital minerals like calcium, magnesium, and potassium, can be used to explain this notable increase. As a result, adding these seeds increased the formulation's total mineral density and raised its ash content [30].

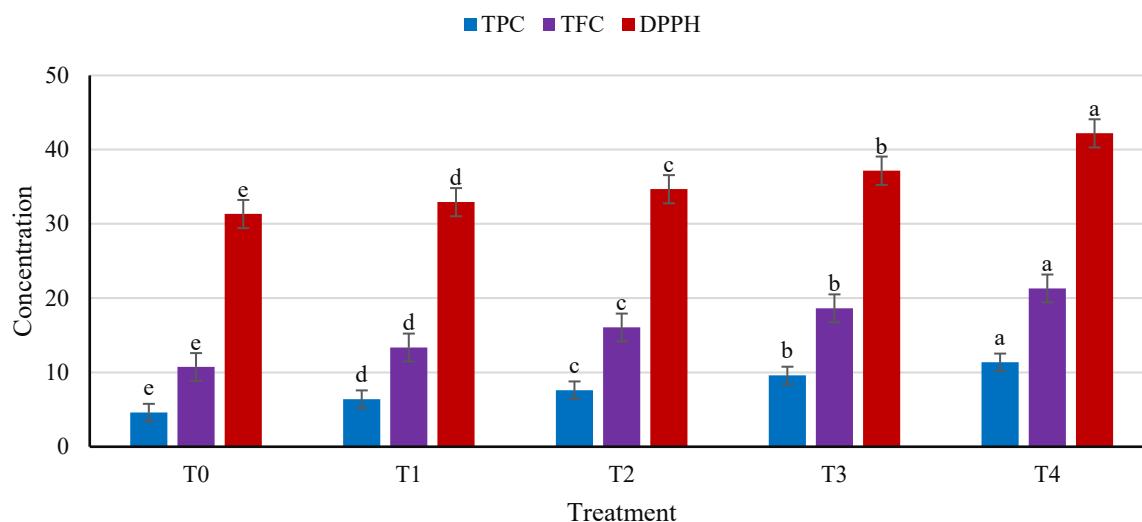
**Table 3.** Proximate composition of beetroot-sweet basil pestils.

| Treatments     | Moisture (g/100 g) | Fat (g/100 g)     | Fiber (g/100 g)   | Protein (g/100 g) | Ash (g/100 g)     |
|----------------|--------------------|-------------------|-------------------|-------------------|-------------------|
| T <sub>0</sub> | $26.6 \pm 0.19^a$  | $7.63 \pm 0.10^a$ | $7.34 \pm 0.20^d$ | $4.26 \pm 0.20^c$ | $1.85 \pm 0.05^c$ |
| T <sub>1</sub> | $25.47 \pm 0.15^b$ | $6.38 \pm 0.15^b$ | $7.96 \pm 0.20^c$ | $4.72 \pm 0.11^c$ | $2.61 \pm 0.11^d$ |
| T <sub>2</sub> | $24.29 \pm 0.17^c$ | $5.79 \pm 0.12^c$ | $8.79 \pm 0.07^b$ | $5.23 \pm 0.69^b$ | $3.11 \pm 0.18^c$ |
| T <sub>3</sub> | $21.81 \pm 0.23^d$ | $4.68 \pm 0.06^d$ | $9.40 \pm 0.19^a$ | $6.43 \pm 0.52^a$ | $3.96 \pm 0.07^b$ |
| T <sub>4</sub> | $19.54 \pm 0.11^e$ | $2.05 \pm 0.09^e$ | $9.73 \pm 0.06^a$ | $7.48 \pm 0.16^a$ | $4.65 \pm 0.09^a$ |

Different superscript letters (a–e) indicate significant differences among the means ( $p < 0.05$ ).

### 3.2. Phytochemical Analysis

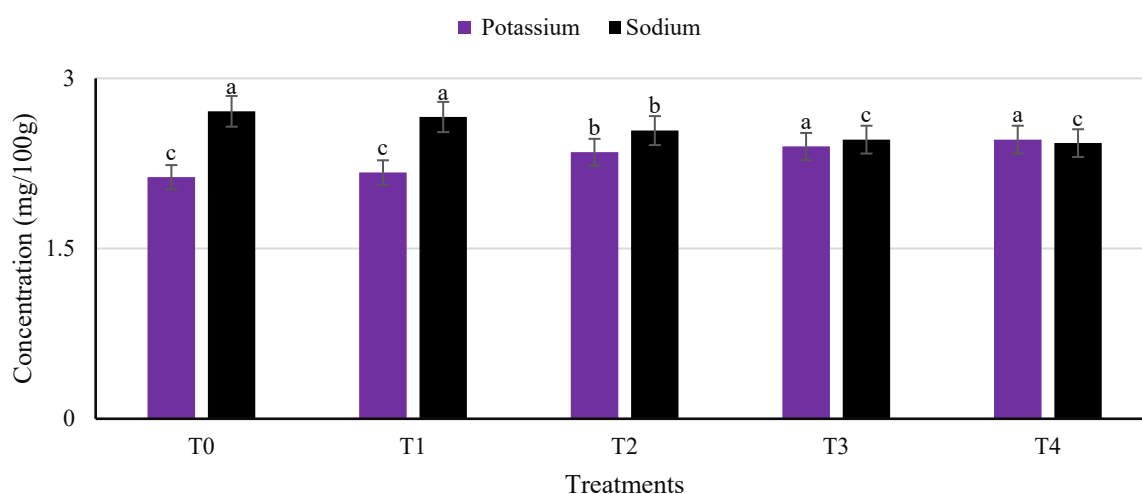
The results of antioxidant analysis revealed that total phenolic content, total flavonoid content and DPPH activity of beetroot pestils increased with increasing concentration of sweet basil leaves, as shown in Figure 1. The total phenolic content increased with the addition of sweet basil powder, likely due to high levels of polyphenols in sweet basil that elevate antioxidant capacity [31]. Results of this study were in agreement with the study of [32] in which antioxidant activity was increased in muffins due to the phenolic compound present in sweet basil leaves. The total flavonoids ranged from 10.74 in T<sub>0</sub> to 21.31 mg CE/100 g in T<sub>4</sub> due to the naturally high flavonoid levels in sweet basil. Sweet basil contains various flavonoids, such as vicenin and orientin, contributing to its antioxidant properties [33]. The results of the present study were comparable with the literature of [18], who reported 19.58 mg CE/100 g total flavonoid content of sweet basil leaves. The DPPH radical scavenging activity of beetroot pestil enriched with sweet basil depicted an increase from 31.34% in T<sub>0</sub> to 42.20% in T<sub>4</sub>. The higher DPPH radical scavenging potential of pestils can be attributed to an increased level of total phenolics and total flavonoids in pestils. Beetroot has been recognized among the most potent antioxidant vegetables because of bioactive compounds like betalains and polyphenols that contribute to its antioxidant potential [34]. In addition to free phenolics and flavonoids content, sweet basil leaves and beetroot contain dietary fiber, which is an abundant source of phenolic acids that might have contributed to the radical scavenging potential of pestils [35]. These results are similar to the findings of research of [36], who analyzed the effect of sweet basil in roselle calyces (*Hibiscus sabdariffa*) drink.



**Figure 1.** Graphical representation of TPC, TFC and DPPH contents of beetroot enriched pestils. Different lowercase letters indicate significant differences among the treatments ( $p < 0.05$ ).

### 3.3. Minerals Composition

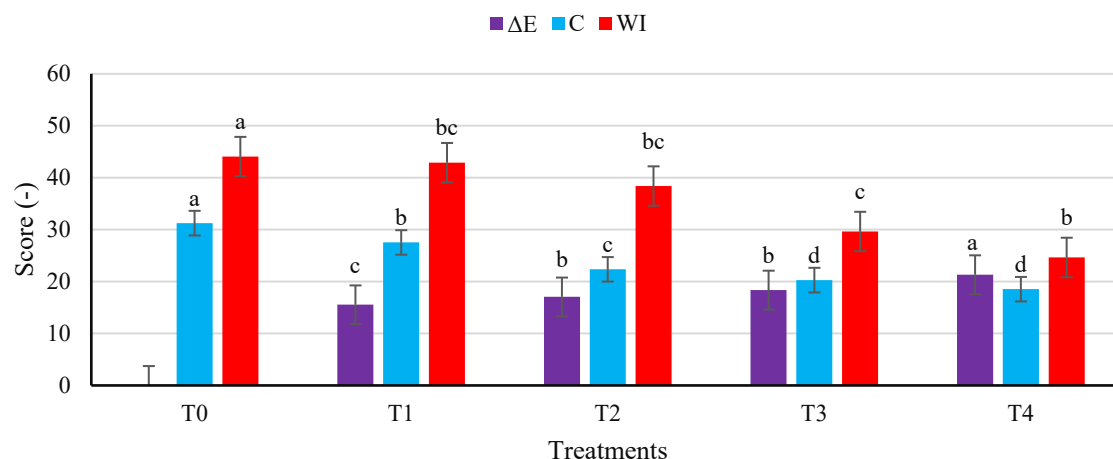
The results of the mineral analysis of the beetroot pestil are presented in Figure 2. The sodium content of the beetroot pestil showed a decreasing trend, ranging from 2.71 mg/100 g to 2.43 mg/100 g with the incorporation of sweet basil. That showed that sweet basil decreases the sodium content of the beetroot pestil. These findings are in line with previously reported literature [37], which showed that the sodium content decreased due to the addition of sweet basil powder in biscuits. The potassium content of the sample ranged from 2.13 mg/100 g to 2.46 mg/100 g. The findings of the present study for potassium contents were found to be slightly greater than the findings of [5], who made sweet basil-fortified biscuits. The observed decrease in sodium and rise in potassium can be attributed to the natural mineral composition of sweet basil leaves, which is relatively low in sodium (4 mg/100 g) and high in potassium (295 mg/100 g) [38]. Additionally, it has been noted that leafy herbs like basil contain ion-exchange and mineral-binding qualities that could help food systems enrich with potassium while lowering sodium retention [37]. Consequently, adding sweet basil improves the nutritional profile of beetroot pestil while also altering the sodium–potassium balance due to its compositional properties. By encouraging a more favorable sodium–potassium ratio, this improvement may enhance cardiovascular health and other possible health benefits.



**Figure 2.** Graphical representation of potassium and sodium contents of beetroot enriched pestils. Different lowercase letters indicate significant differences among the treatments ( $p < 0.05$ ).

### 3.4. Color Analysis

Figure 3 shows the results of the color analysis of the beetroot-enriched pestils. It is observed that with increasing enrichment, the  $\Delta E$  value increases from  $0.00 \pm 0.00$  in T<sub>0</sub> to  $21.31 \pm 0.10$  in T<sub>4</sub>, implying a greater difference in color with increasing enrichment. The highest  $\Delta E$  value was found in T<sub>4</sub> (20% sweet basil powder). The reason for the change in color could be due to the presence of pigments and other ingredients from sweet basil powder in beetroot pestil. These results are similar to that of [39], which showed an increasing trend in color difference in baguette bread during staling. However, there was progressive decrease in chroma value from  $31.25 \pm 0.10$  for T<sub>0</sub> to  $18.55 \pm 0.31$  for T<sub>4</sub>. This could be due to the mask effect caused by pigments in sweet basil powder. This has been reported by [40]. Also, there was progressive decrease in whiteness index from  $44.07 \pm 0.01$  for T<sub>0</sub> to  $24.64 \pm 0.22$  for T<sub>4</sub>. The reduction in whiteness index signifies that the enriched pestils were darker and might be due to change in pigments during the enrichment process and hot air-drying process.



**Figure 3.** A representation of change in color, chroma and whiteness index of beetroot enriched pestils. Different lowercase letters indicate significant differences among the treatments ( $p < 0.05$ ).

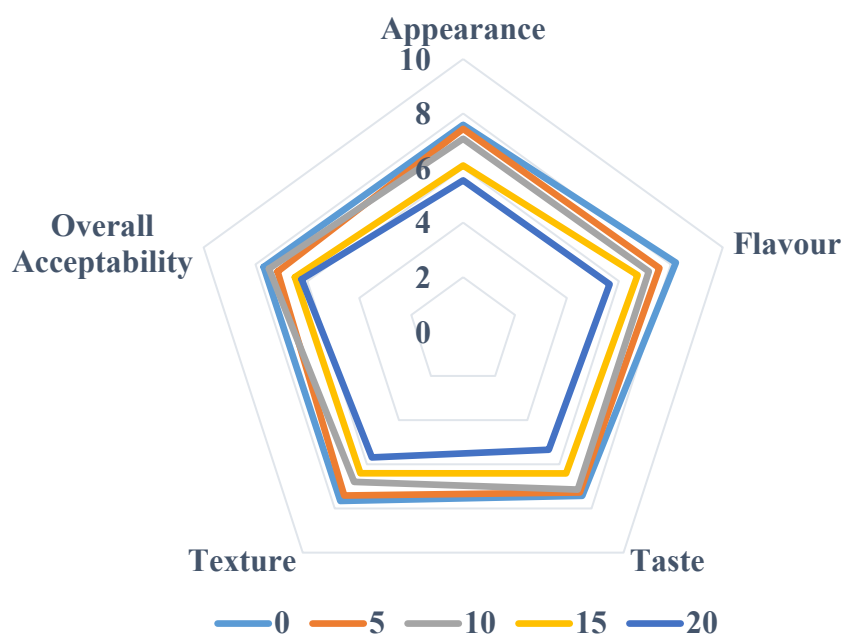
### 3.5. Sensory Evaluation

The sensory assessment of beetroot sweet basil pestil was conducted using a panel of twenty semi-trained graduate students (ages 20 to 30 years). It was based on appearance, flavor (sweet, sour), taste (sweet, bitter), texture (soft, hard), and overall acceptability. The results are shown in Table 4 and Figure 4. Beetroot significantly affected the pestil's sensory attributes. The pestil sample containing 20% sweet basil exhibited a bitter taste, leading to lower ratings for taste and overall acceptability. It may be due to compounds like eugenol, tannins and linalool causing bitterness, astringency and overpowering aromas. The texture of pestil became harder with increases in sweet basil powder. These results are related to literature in which they study that the texture of cookies becomes hard by incorporation of a large amount of sweet basil powder [41]. This may be likely due to the high fiber content of basil leaves and beetroot which binds water, thereby reducing availability of free water and altering the pestil's structure. This limits matrix flexibility and results in a denser texture in pestil [42]. The flavor also diminished with the addition of sweet basil compared to the control. This may be due to strong herbal notes of sweet basil that may overshadow the beetroot's natural flavors, resulting in a decreased flavor appeal due to intensified bitterness [43]. Likewise, acceptance results showed decreasing trend as the sweet basil was added. The results indicated that beetroot pestil with 5% sweet basil was acceptable from the consumer perspective. Therefore, this pestil is selected for efficacy research.

**Table 4.** Sensory evaluation of beetroot enriched pestils.

| Treatments     | Appearance               | Flavor                    | Taste                     | Texture                  | Overall acceptability     |
|----------------|--------------------------|---------------------------|---------------------------|--------------------------|---------------------------|
| T <sub>0</sub> | 7.59 ± 0.15 <sup>a</sup> | 8.18 ± 0.18 <sup>a</sup>  | 7.42 ± 0.10 <sup>a</sup>  | 7.65 ± 0.12 <sup>a</sup> | 7.68 ± 0.12 <sup>a</sup>  |
| T <sub>1</sub> | 7.44 ± 0.10 <sup>a</sup> | 7.55 ± 0.13 <sup>ab</sup> | 7.28 ± 0.12 <sup>ab</sup> | 7.41 ± 0.20 <sup>a</sup> | 7.14 ± 0.03 <sup>a</sup>  |
| T <sub>2</sub> | 7.07 ± 0.13 <sup>b</sup> | 7.15 ± 0.03 <sup>b</sup>  | 7.14 ± 0.04 <sup>b</sup>  | 6.79 ± 0.12 <sup>b</sup> | 7.48 ± 0.08 <sup>bc</sup> |
| T <sub>3</sub> | 6.09 ± 0.10 <sup>c</sup> | 6.72 ± 0.09 <sup>c</sup>  | 6.41 ± 0.10 <sup>c</sup>  | 6.40 ± 0.12 <sup>b</sup> | 6.48 ± 0.30 <sup>c</sup>  |
| T <sub>4</sub> | 5.55 ± 0.11 <sup>d</sup> | 5.64 ± 0.34 <sup>c</sup>  | 5.34 ± 0.07 <sup>d</sup>  | 5.68 ± 0.16 <sup>c</sup> | 6.22 ± 0.09 <sup>d</sup>  |

Different superscript letters (a–c) indicate significant differences among the means ( $p < 0.05$ ).



**Figure 4.** Effect of different concentration of sweet basil powder on sensory analysis of beetroot pestil

### 3.6. Serum Lipid Profile

The effect of beetroot pestil on the serum lipid profile (LDL, HDL, total cholesterol, and TG) in animals was evaluated and results are presented in Table 5. The results showed a significant relationship between the treatment group and the time interval of the study. LDL, total cholesterol, and triglyceride levels decreased progressively from baseline to the 30th day in beetroot-sweet basil pestils groups, while HDL levels improved significantly over the same period. At baseline, all groups ( $G_1$ ,  $G_2$ ,  $G_3$ ) exhibited elevated LDL and total cholesterol levels compared to negative control group ( $G_0$ ) that confirmed successful induction of hyperlipidemia. HDL levels were relatively lower in  $G_2$  and  $G_3$  indicating disturbed lipid metabolism associated with hyperlipidemia.

By the 15th day, a modest decline in serum LDL, total cholesterol, triglycerides and an increase in HDL levels was observed in the intervention groups, compared to baseline whereas, negative control group  $G_0$  has shown a negligible change.

After thirty days of intervention the effect became more pronounced. A gradual increase in HDL levels (mg/dL) was observed in the treatment groups compared to baseline and control groups. In the group  $G_2$ , HDL increased from  $29.8 \pm 3.70$  at baseline to  $33.2 \pm 2.77$  at day 30, reflecting an approximate 11.4% increase in serum HDL. In group  $G_3$  HDL increased from  $30 \pm 3.67$  at baseline to  $34.6 \pm 2.60$  at day 30, corresponding to an approximate 15.3% increase. The findings of the present research are correlated with prior reported literature [44], who suggested that beetroot is effective against hypercholesterolemia and improves serum HDL levels. They fed diabetic rats with 6% beetroot powder and 6% beetroot extract and reported an increase in serum HDL levels at 7.75 and 9.34%, respectively when compared with positive control.

By day 30, LDL cholesterol (mg/dL) levels declined significantly in the intervention groups compared to baseline LDL values and control groups. In group  $G_2$ , LDL decreased from  $165.2 \pm 2.94$  at baseline to  $160.0 \pm 3.08$  on day 30, showing a 3.15% reduction. In group  $G_3$ , a sharper decline in LDL levels, from  $164.8 \pm 2.38$  to  $156.4 \pm 4.06$  was recorded, corresponding to a 5.10% reduction. The reduction in LDL may be attributed to the fact that beetroot contains betalains and nitrates which combine with the polyphenols in basil, which help to reduce lipid oxidation and improve lipid metabolism [45]. This also demonstrates an obvious dose-dependent effect, as the higher supplementation level in  $G_3$  produced a greater reduction in LDL compared to  $G_2$ .

Similarly, the maximum cholesterol (mg/dL) reduction was recorded in  $G_3$  from  $221.6 \pm 2.70$  at baseline to  $206.4 \pm 5.50$  on day 30, followed by group  $G_2$  from  $211 \pm 3.39$  at baseline to  $199.2 \pm 7.01$  at 30th day of intervention.  $G_0$  and  $G_1$  demonstrated lower mean cholesterol reduction ( $179 \pm 14.88$  to  $177.8 \pm 15.25$ ) and ( $205.4 \pm 4.56$  to  $204 \pm 6.32$ ), respectively. The results obtained from  $G_2$  and  $G_3$  were approximately similar and both groups decreased cholesterol to a considerable level. The reduction is due to the presence of fiber and the addition of pectin which also reduces the cholesterol level. The fiber absorbs the cholesterol and takes it away from the body through urine concentration [46].

Triglyceride (TG) levels (mg/dL) also significantly decreased in the treatment groups by day 30 compared to baseline. In group  $G_2$ , TG decreased from  $165.2 \pm 3.70$  at baseline to  $157.0 \pm 1.34$  at day 30, corresponding to a

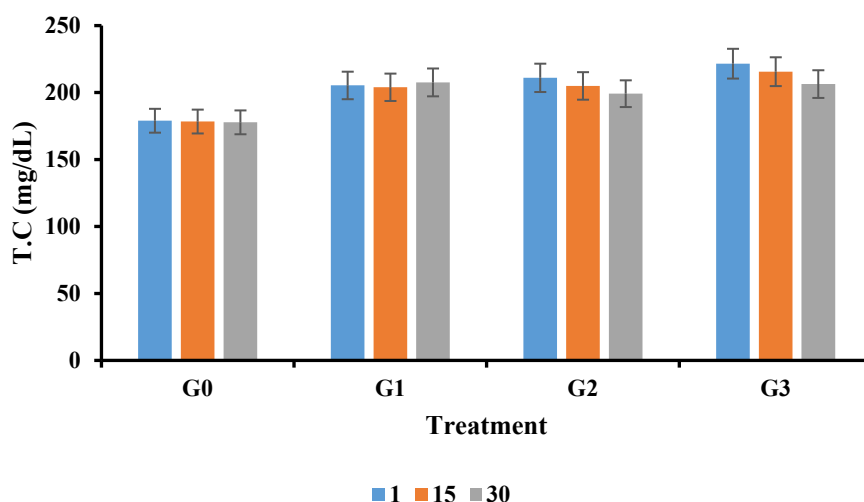
4.96% reduction. Similarly, in group G<sub>3</sub>, TG levels declined from  $165.0 \pm 3.87$  to  $154.8 \pm 5.06$  recorded at baseline and 30th day of intervention, representing a 6.18% decrease. The magnitude of decline was more pronounced in G<sub>3</sub>, suggesting a stronger lipid-lowering effect with higher levels of beetroot-sweet basil pestils supplementation. These findings are in agreement with another study that investigated the administration of 1.2 g/kg extract of beetroot could provide an important alternative strategy to control dyslipidemia and reduce triglyceride by 19% in diabetic rats [44]. No significant change was observed in the drop of TG levels in G<sub>0</sub> ( $137.2 \pm 1.92$ – $138.8 \pm 1.48$ ). A slightly considerable change was witnessed in G<sub>1</sub> from  $153.2 \pm 4.14$  to  $151.4 \pm 2.88$ .

**Table 5.** Effect of beetroot-sweet basil pestils on HDL, LDL, total cholesterol and triglycerides in rats.

| Days | Treatment      | HDL<br>(mg/dL)        | LDL<br>(mg/dL)        | Total Cholesterol<br>(mg/dL) | TG<br>(mg/dL)          |
|------|----------------|-----------------------|-----------------------|------------------------------|------------------------|
| 0    | G <sub>0</sub> | $38.2 \pm 2.58^a$     | $104.8 \pm 3.56^d$    | $179 \pm 14.88^c$            | $137.2 \pm 1.92^c$     |
|      | G <sub>1</sub> | $38.6 \pm 2.07^c$     | $146.6 \pm 2.30^c$    | $205.4 \pm 4.56^{ab}$        | $153.2 \pm 4.14^d$     |
|      | G <sub>2</sub> | $29.8 \pm 3.70^a$     | $165.2 \pm 2.94^a$    | $211 \pm 3.39^{ab}$          | $165.2 \pm 3.70^a$     |
|      | G <sub>3</sub> | $30 \pm 3.67^c$       | $164.8 \pm 2.38^a$    | $221.6 \pm 2.70^a$           | $165 \pm 3.87^a$       |
| 15   | G <sub>0</sub> | $37.8 \pm 2.28^a$     | $106.2 \pm 3.59^d$    | $178.4 \pm 15.07^c$          | $138 \pm 2.12^c$       |
|      | G <sub>1</sub> | $37.6 \pm 2.07^{ab}$  | $147.6 \pm 2.19^c$    | $204 \pm 6.32^{ab}$          | $153.6 \pm 2.96^{cd}$  |
|      | G <sub>2</sub> | $31.4 \pm 3.20^{bc}$  | $163.6 \pm 3.64^a$    | $205 \pm 6.20^{ab}$          | $162.6 \pm 3.57^{ab}$  |
|      | G <sub>3</sub> | $32.8 \pm 3.76^{abc}$ | $160.4 \pm 1.81^{ab}$ | $215.6 \pm 3.50^{ab}$        | $160.2 \pm 4.43^{abc}$ |
| 30   | G <sub>0</sub> | $38.4 \pm 2.70^a$     | $107.2 \pm 2.48^d$    | $177.8 \pm 15.25^{ab}$       | $138.8 \pm 1.48^c$     |
|      | G <sub>1</sub> | $37.6 \pm 2.50^{ab}$  | $148.4 \pm 3.50^c$    | $207.6 \pm 7.26^{ab}$        | $151.4 \pm 2.88^{cd}$  |
|      | G <sub>2</sub> | $33.2 \pm 2.77^{abc}$ | $160 \pm 3.08^{ab}$   | $199.2 \pm 7.01^b$           | $157.0 \pm 1.34^{bcd}$ |
|      | G <sub>3</sub> | $34.6 \pm 2.60^{abc}$ | $156 \pm 4.06^b$      | $206.4 \pm 5.50^{ab}$        | $154.8 \pm 5.06^{cd}$  |

Different superscript letters (a–d) indicate significant differences among the means ( $p < 0.05$ ).

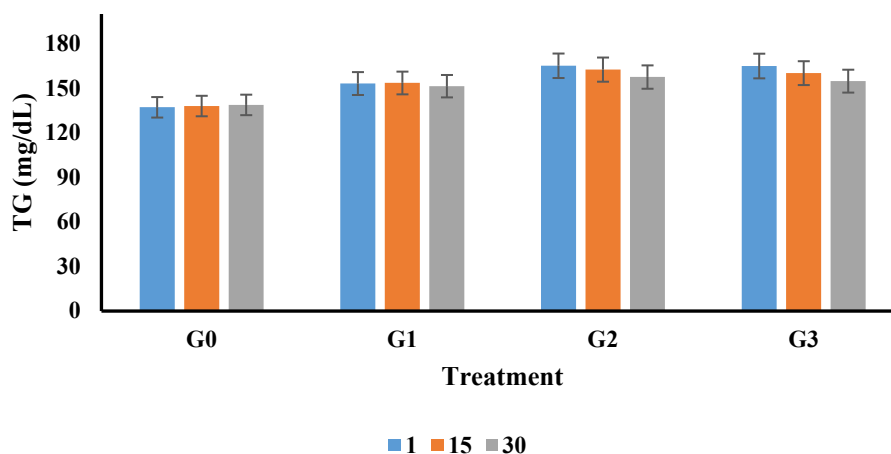
Maximum cholesterol reduction was recorded in G<sub>3</sub> ( $221.6 \pm 2.70$ – $206.4 \pm 5.50$ ) and then in G<sub>2</sub> ( $211 \pm 3.39$ – $199.2 \pm 7.01$ ) as shown in Table 5 and Figure 5. G<sub>0</sub> and G<sub>1</sub> demonstrated lower mean cholesterol reduction;  $179 \pm 14.88$ – $177.8 \pm 15.25$  and  $205.4 \pm 4.56$ – $204 \pm 6.32$ , respectively. Results obtained from G<sub>2</sub> and G<sub>3</sub> were approximately similar and both groups decreased cholesterol to a considerable level.



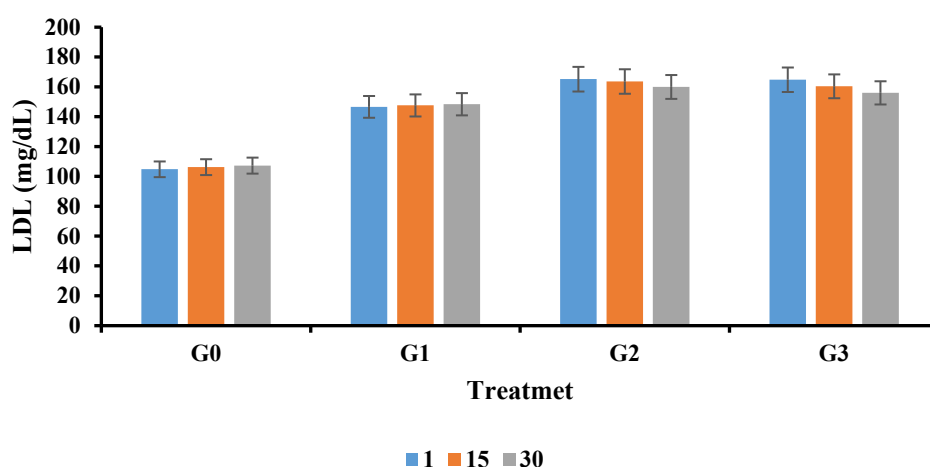
**Figure 5.** Changes in serum total cholesterol (TC) levels during the experimental period (0, 15, and 30 days).

No significant change was observed in drop of TG levels in G<sub>0</sub> ( $137.2 \pm 1.92$ – $138.8 \pm 1.48$ ). Slightly considerable change was witnessed in both G<sub>1</sub> and G<sub>2</sub>;  $153.2 \pm 4.14$ – $151.4 \pm 2.88$  and  $165.2 \pm 3.70$ – $157 \pm 1.34$ , respectively. On the other hand, maximal reduction in TG contents was recorded in G<sub>3</sub>, which was from  $165 \pm 3.87$ – $154.8 \pm 5.06$ . This leads to the conclusion that the G<sub>3</sub> was most successful in attenuating the levels of TG in rats, as shown in Figure 6.

Increase the LDL level was documented in disease group G<sub>0</sub> and G<sub>1</sub>. This increased ranged from  $104.8 \pm 3.56$ – $107.2 \pm 2.48$  in G<sub>0</sub> and  $146.6 \pm 2.30$ – $148.4 \pm 3.50$  in G<sub>1</sub>. G<sub>2</sub> diminished LDL numeric from  $165.2 \pm 2.94$ – $160 \pm 3.08$  and in G<sub>3</sub> the values shrunk from  $164.8 \pm 2.38$ – $156 \pm 4.06$ , as shown in Figure 7.

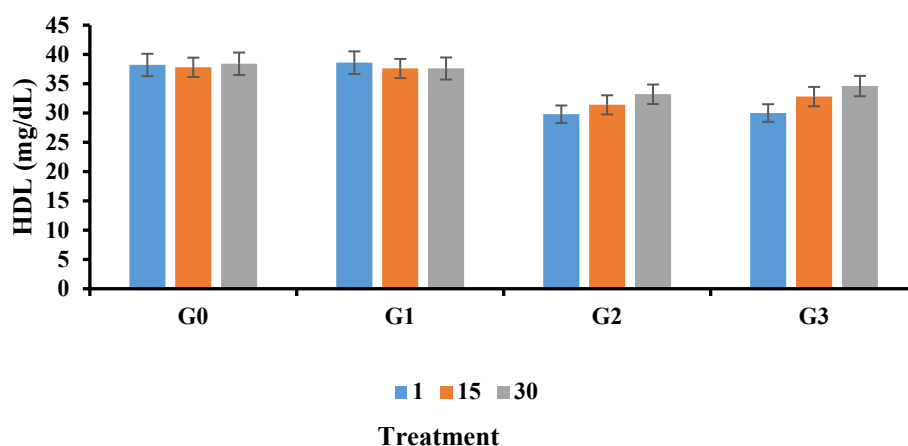


**Figure 6.** Changes in serum triglyceride (TG) levels during the experimental period (0, 15, and 30 days).



**Figure 7.** Changes in serum low-density lipoprotein cholesterol (LDL-C) levels during the experimental period (0, 15, and 30 days).

The HDL contents improved in 2 groups (G<sub>2</sub>, G<sub>3</sub>). However, this upsurge was minimal in G<sub>0</sub> (0.5%) where the HDL levels increased from  $38.2 \pm 2.58$ – $38.4 \pm 2.70$ . G<sub>2</sub> showed a moderate increase from  $29.8 \pm 3.70$ – $33.2 \pm 2.77$ . In G<sub>3</sub> maximum escalation in HDL was observed from  $30 \pm 3.67$ – $34.6 \pm 2.60$ . On the other hand, G<sub>1</sub> disease group showed a highly significant decrease in HDL levels from  $38.6 \pm 2.07$ – $37.6 \pm 2.50$ . This obtained data concluded that the increment of HDL in both groups (G<sub>2</sub> and G<sub>3</sub>) is to a considerable level, as shown in Figure 8.



**Figure 8.** Changes in serum high-density lipoprotein cholesterol (HDL-C) levels during the experimental period (0, 15, and 30 days).

#### 4. Conclusions

This study provides evidence that beetroot pestils enriched with sweet basil leaf powder not only improved nutritional and antioxidant properties but also exerted significant lipid-modulating effects in hyperlipidemic rats. Supplementation, particularly at 150 mg/kg bw/day for 30 days, was associated with increased HDL and reductions in total cholesterol, LDL, and triglycerides, indicating a favorable impact on serum lipid profiles. This study indicates that beetroot-sweet basil pestils could possibly serve as an additional dietary approach for enhancing lipid parameters in hyperlipidemic rats. Nonetheless, considering the limited number of participants in the study and the short period used for the interventions (30 days), these results should only be viewed as preliminary and need further investigation.

#### 5. Recommendation

- *Beta vulgaris* L. should be consumed as a functional food as it is also cost-effective therapy to handle lifestyle related morbidities.
- *Ocimum basilicum* L. contains essential minerals and vitamins so this plant can also be used in vitamins and minerals deficiency in need
- Medicinal plants show the therapeutic effect so these should be consumed in daily diet for the treatment of different complicated disease.
- Fruit leather should be preferred over jam and jellies due to nutritional composition so that it can be used as nutrient-dense snack.
- Beetroot and sweet basil are rich source of bioactive components that reduces risk of hypertension, cardiovascular disease, lipid accumulation and obesity.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available from the corresponding author upon reasonable request.

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