"Synergistic Effects of Methanolic Extract of Ginseng and Yogurt on Weight Reduction and Metabolic Health: An Experimental Study in a High-Fat Diet-Induced Obesity Model"

M. Pharm (Masters) Thesis Report

[In the partial fulfillment of the requirements for the degree of Master of Pharmacy]

Submitted To

Department of Pharmacy

Faculty of Health and Life Sciences

Daffodil International University

Submitted By

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APPROVAL

This is to certify that the thesis titled ***"Synergistic Effects of Methanolic Extract of Ginseng and Yogurt on Weight Reduction and Metabolic Health: An Experimental Study in a High-Fat Diet-Induced Obesity Model"*** has been submitted to the Department of Pharmacy, Faculty of Health and Life Sciences, Daffodil International University, in partial fulfillment of the requirements for the degree of Master of Pharmacy. The thesis has been examined and approved as to its academic merit, style, and content.

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With the utmost humility and gratitude, I begin by offering my heartfelt thanks to the Almighty ALLAH, whose infinite mercy and blessings have been my constant source of strength, peace, and perseverance throughout this journey. Alhamdulillah, for guiding me through every challenge and enabling the successful completion of this thesis.

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DECLARATION

I, Md. Abid Hossain, hereby declare that the thesis work titled ***"Synergistic Effects of Methanolic Extract of Ginseng and Yogurt on Weight Reduction and Metabolic Health: An Experimental Study in a High-Fat Diet-Induced Obesity Model"*** has been conducted entirely through my own efforts and is based on observations made under the supervision of **Prof. Dr. Sharifa Sultana**, Associate Professor and Associate Head of the Department of Pharmacy, Daffodil International University. This work is submitted in partial fulfillment of the requirements for the degree of Master of Pharmacy.

I further declare that this thesis has not been submitted previously, in whole or in part, to any other university or institution for the award of any degree, diploma, or professional qualification, except as specified within the document.

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DEDICATION

Thank You

In the name of Allah, the Most Gracious, the Most Merciful,

All praise and thanks to Allah, whose blessings and guidance have enabled me to complete this work.

I dedicate this thesis to my beloved family for their unwavering love, support, and prayers, and to my esteemed teachers, whose wisdom and encouragement have inspired and guided me throughout this journey.

May Allah bless everyone who has contributed to my success.

Md. Abid Hossain

ABSTRACT

Background

Obesity and its associated metabolic disorders have become a major public health concern, necessitating the development of safe and effective therapeutic options. Natural compounds, such as ginseng and probiotics, are increasingly being explored for their potential to improve metabolic health and manage obesity.

Objective

This study aimed to evaluate the synergistic effects of methanolic ginseng extract and probiotics on weight management and lipid metabolism in a high-fat diet-induced obesity model. Additionally, the presence of ginsenosides in the ginseng extract was confirmed to ensure the authenticity and therapeutic potential of the bioactive compounds.

Methodology

The study employed high-performance liquid chromatography (HPLC) to confirm the presence of ginsenosides in methanolic ginseng extract. Subsequently, an animal model study was conducted using high-fat diet-induced obesity in mice. The mice were divided into experimental groups receiving standalone and combination therapies of ginseng and probiotics, as well as a control and standard drug group. Weight changes, lipid profiles, and metabolic parameters were evaluated over the study period, and statistical analyses were conducted to determine treatment efficacy.

Results and Discussion

The combination therapy of ginseng and probiotics showed superior results in weight reduction, increased HDL levels, reduced triglycerides, and stabilized LDL and total cholesterol levels compared to standalone treatments and the standard pharmacological intervention (Orlistat). The use of HPLC ensured the presence of therapeutic ginsenosides in the ginseng extract, strengthening the validity of the results. The findings suggest a synergistic effect between ginseng and probiotics in improving metabolic health, highlighting their potential for natural obesity management.

Conclusion

The study demonstrates that the combination of ginseng and probiotics is a promising natural therapeutic approach for managing obesity and related metabolic disorders. Future studies should focus on optimizing dosage, exploring long-term effects, and validating these findings in clinical trials to establish the efficacy and safety of this combination therapy for widespread use.

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Chapter One

Introduction

1.1. Introduction

Obesity has become a critical global health issue, with over 650 million adults affected worldwide, as reported by the World Health Organization (2020). This complex condition is often accompanied by a range of comorbidities such as type 2 diabetes, cardiovascular diseases, and certain cancers, placing immense strain on healthcare systems globally. Despite various management strategies, including dietary modifications, physical activity programs, and pharmacological interventions like Orlistat, long-term success in managing obesity remains elusive. While pharmacological treatments like Orlistat help in weight reduction by inhibiting gastrointestinal lipases, they are not without challenges [1]. Orlistat, being a synthetic drug, is associated with adverse effects such as gastrointestinal discomfort, nutrient malabsorption, and potential toxicity with prolonged use. These side effects, combined with concerns about long-term adherence, highlight the need for alternative therapeutic options.

Thus, there is an increasing focus on identifying novel, safe, and effective strategies that not only aid in weight reduction but also offer broader metabolic health benefits with minimal adverse effects. Natural remedies, particularly those derived from plant-based sources, have garnered attention due to their multifaceted therapeutic profiles. Ginseng (Panax ginseng), a traditional herbal medicine, is rich in bioactive compounds, particularly ginsenosides, which have been shown to offer significant metabolic benefits. Among these, ginsenoside Rg1 has emerged as a key player in the regulation of lipid metabolism, fat oxidation, and energy expenditure via activation of the AMP-activated protein kinase (AMPK) pathway. Furthermore, Rg1 has been shown to inhibit the NLRP3 inflammasome, which is linked to inflammation-induced obesity, including the downregulation of lipid biosynthesis regulators and reduction of oxidative stress, both of which are critical mechanisms in the pathophysiology of obesity [2] [3]. Beyond its ginsenoside content, ginseng also contains approximately 8.98% soluble dietary fiber (SDF), which enhances its value as a prebiotic [4]. Prebiotics are compounds that stimulate the growth of beneficial gut bacteria, which play an essential role in metabolic health. In particular, SDF in ginseng helps to foster gut microbiota that supports weight management by improving satiety, regulating carbohydrate digestion, and reducing inflammatory responses associated with obesity.

Recent studies have highlighted the complementary benefits of combining prebiotics with probiotics—beneficial microorganisms found in foods like yogurt. Probiotics contribute to improved gut microbiota composition, reduced systemic inflammation, and enhanced metabolic function, thereby supporting anti-obesity effects. When combined with a prebiotic

like ginseng's SDF, probiotics may achieve enhanced, synergistic outcomes, including improved lipid metabolism and greater reduction in adiposity. Notably, the addition of ginseng-enriched yogurt has been shown to elevate antioxidant activity, suggesting that functional foods incorporating ginseng and probiotics could have therapeutic roles in obesity management [5].

The methanolic extract of ginseng, which contains a variety of ginsenosides including Rg1, provides a comprehensive therapeutic approach to obesity [3]. This extract has the potential to serve as an effective, natural alternative to synthetic drugs like Orlistat, which, while beneficial in reducing weight, may lead to toxicity and adverse gastrointestinal effects. Additionally, ginseng can be combined with probiotics, whose beneficial effects on gut microbiota and metabolism have been well documented. This combined treatment offers a synergistic effect that may enhance the therapeutic benefits of both agents, potentially reducing the adverse effects associated with Orlistat and improving both weight reduction and lipid profiles in a safer and more holistic manner.

Given the synthetic nature of Orlistat and its associated toxicity, the primary aim of this study is to identify a novel therapeutic strategy that combines ginseng and probiotics for weight reduction and the improvement of lipid profiles. This study aims to evaluate the synergistic effects of ginsenoside Rg1 from ginseng root extract and probiotics in a high-fat diet-induced obesity model, comparing its efficacy with Orlistat as the standard treatment. Specifically, we assessed weight variations on the 1st day, 7th day, and 14th day of treatment, and measured lipid profiles, including total cholesterol, HDL, LDL, and triglycerides at each time point. The hypothesis is that the combination of ginseng and probiotics will not only provide comparable or superior results to Orlistat in improving lipid metabolism but will also offer a safer alternative with fewer side effects.

1.2. Plant Profile

Ginseng refers to perennial plants belonging to the genus Panax within the Araliaceae family, encompassing approximately 12 species [6]. These herbs are renowned for their medicinal properties, particularly the fleshy roots, which have been integral to traditional medicine practices in East Asia and North America.

Physical Description: Ginseng plants typically reach heights of up to 60 cm (about 24 inches) [7]. They feature compound leaves arranged in a whorl, each comprising five leaflets with serrated edges.

Figure 1: Panax Ginseng Root
Image Courtesy: Google Image

The plants produce small greenish flowers clustered in umbels, which, upon maturation, yield red berry-like fruits. The most distinctive feature is the thick, often forked root, which bears a resemblance to the human form, contributing to its name—Panax meaning "all-healing" in Greek, and "ginseng" derived from the Chinese term rénshēn, meaning "person root [8]."

1.2.1. Species and Distribution

Asian Ginseng (Panax ginseng): Native to regions such as Manchuria and Korea, this species has been utilized for centuries in traditional Chinese, Korean, and Japanese medicine.

American Ginseng (Panax quinquefolius): Indigenous to North America, ranging from Quebec and Manitoba in Canada to the Gulf of Mexico in the United States. This species is also employed in traditional medicine and is extensively cultivated for export, particularly to Asian markets [8].

1.2.2. Phytochemical Constituents of Ginseng

Ginseng (Panax species) contains a rich variety of bioactive phytochemicals, primarily responsible for its therapeutic properties. The key constituents include:

1.2.2.1. Ginsenosides

Ginsenosides are the major active components of ginseng, belonging to the triterpenoid saponin family. These compounds are classified into two main types: protopanaxadiols (PPD) and protopanaxatriols (PPT), based on their aglycone structures.

- Examples of PPD ginsenosides: Rb1, Rb2, Rc, and Rd.
- Examples of PPT ginsenosides: Re, Rg1, Rg2, and Rh1 [9].

Ginsenosides contribute to ginseng's anti-obesity effects through their roles in energy metabolism, anti-inflammatory activity, and adipogenesis inhibition.

1.2.2.2. Polysaccharides

These complex carbohydrates have immunomodulatory and antioxidant properties, playing supportive roles in metabolic regulation.

1.2.2.3. Polyacetylenes

Panaxynol and panaxydol are examples of polyacetylenes found in ginseng. They exhibit anti-inflammatory, cytotoxic, and lipid-lowering effects.

1.2.2.4. Phenolic Compounds

Ginseng contains phenolic compounds that contribute to its antioxidant activities, which help mitigate oxidative stress associated with obesity.

1.2.2.5. Vitamins and Minerals

Essential micronutrients in ginseng provide additional health benefits, supporting overall metabolic and physiological health.

1.2.2.6. Amino Acids and Peptides

These constituents aid in protein metabolism and cellular repair, indirectly supporting anti-obesity mechanisms [9].

1.2.3. Medicinal Uses

Ginseng roots are esteemed for their adaptogenic properties, believed to enhance the body's resilience to stress, boost energy levels, and improve overall well-being. In traditional medicine, ginseng is often consumed as a tea or in powdered form and is associated with benefits such as improved cognitive function, immune support, and anti-inflammatory effects and cardiovascular function. However, scientific studies on these health benefits have yielded mixed results, and further research is necessary to substantiate these claims [10], [11].

1.2.3. Cultivation and Conservation

Due to high demand, wild ginseng populations have declined, leading to increased cultivation efforts. American ginseng, for instance, is primarily cultivated in regions like Wisconsin and Ontario, with a significant portion of the harvest exported to Asia. Conservation measures are in place to protect wild ginseng species from overharvesting and habitat loss.

In summary, ginseng encompasses a group of perennial herbs with significant cultural and medicinal importance across various regions. Its distinctive morphology and reputed health benefits continue to make it a subject of interest in both traditional medicine and modern scientific research [8].

Chapter Two

Literature Review

2. Literature Review

2.1. Obesity and Its Management

Obesity is a global health issue affecting over 650 million adults, contributing to comorbidities such as type 2 diabetes, cardiovascular diseases, and certain cancers [11]. Current pharmacological interventions, such as Orlistat, offer some efficacy in weight management but are often associated with adverse effects like gastrointestinal discomfort and nutrient malabsorption, limiting their long-term use [12]. This has driven the search for alternative therapeutic strategies that are both effective and safer.

2.2. The Role of Natural Compounds in Obesity Management

Natural compounds, particularly those derived from plants, have garnered significant attention for their potential in addressing obesity and its associated metabolic dysfunctions. Among these, ginseng (Panax species) stands out due to its diverse bioactive constituents and multifaceted therapeutic effects [12].

2.3. Ginseng (Panax Species)

Ginseng, a perennial herb of the genus Panax, has been used for centuries in traditional medicine for its adaptogenic, metabolic, and immunomodulatory properties. Its medicinal value is attributed to its rich phytochemical profile, particularly ginsenosides, polysaccharides, polyacetylenes, and phenolic compounds [13].

2.3.1. Phytochemical Constituents of Ginseng

Ginseng is a reservoir of bioactive compounds that contribute to its therapeutic potential:

- Ginsenosides: These steroidal saponins are the most studied components, classified into protopanaxadiols (e.g., Rb1, Rb2, Rc) and protopanaxatriols (e.g., Rg1, Re, Rg2) [14]. Ginsenosides have been shown to modulate energy metabolism, reduce inflammation, and inhibit adipogenesis, making them key players in obesity management [15].
- Polysaccharides: Known for their antioxidant and immunomodulatory effects, polysaccharides play a supportive role in regulating metabolic health.
- Polyacetylenes and Phenolic Compounds: These contribute to ginseng's anti-inflammatory and lipid-lowering effects.

- Vitamins, Minerals, Amino Acids, and Peptides: These constituents support overall metabolic health and repair mechanisms [13]..

2.4. Pharmacological Properties of Ginsenosides

Ginsenosides, the primary bioactive components in ginseng, exhibit diverse pharmacological properties:

- Anti-inflammatory Effects: Ginsenosides suppress pro-inflammatory cytokines such as TNF-α and IL-6 and reduce inflammatory responses in diseases like arthritis and colitis [16].
- Anti-tumor Effects: They promote apoptosis in cancer cells, inhibit proliferation, and enhance sensitivity to chemotherapy, making them promising agents in cancer treatment [17] [18].
- Neuroprotective Effects: Ginsenosides improve cognitive function, reduce neuronal damage, and enhance neurogenesis. These effects extend to metabolic health by improving insulin sensitivity and reducing blood glucose levels [19].

2.5. The Synergistic Potential of Ginseng and Probiotics

Combining ginseng with probiotics, such as those found in yogurt, introduces a novel approach to addressing obesity and metabolic dysfunctions. Probiotics improve gut microbiota composition, reduce systemic inflammation, and enhance lipid metabolism. When paired with ginseng, particularly its soluble dietary fiber (SDF) and ginsenosides, the synergistic effects amplify weight reduction and improve metabolic outcomes. Functional foods incorporating both agents show promise in managing obesity holistically [20].

2.6. Hypothesis and Research Gap

Although ginseng and probiotics have been individually studied for their anti-obesity effects, limited research exists on their combined efficacy. The methanolic extract of ginseng, rich in ginsenosides like Rg1, when paired with probiotics, offers a natural, effective alternative to synthetic drugs like Orlistat. This study aims to explore this synergy in a high-fat diet-induced obesity model, evaluating its effects on weight, lipid profiles, and systemic inflammation compared to Orlistat.

Chapter Three

Objective f The Study

3. Objective of the Study

1. **RPHPLC Analysis**
   - Identify and quantify key bioactive compounds in ginseng extract, particularly ginsenoside Rg1, through RPHPLC analysis.
2. **Investigate Synergistic Effects**
   - Evaluate the combined effects of ginsenoside Rg1 from ginseng root extract and probiotics on:
     - Weight reduction.
     - Improvement of lipid profiles in a high-fat diet-induced obesity model.
3. **Weight Variation Assessment**
   - Measure weight changes at specific time points:
     - Day 1.
     - Day 7.
     - Day 14.
4. **Lipid Profile Evaluation**
   - Compare the effects of the ginseng-probiotic combination and Orlistat on:
     - Total cholesterol.
     - High-density lipoprotein (HDL).
     - Low-density lipoprotein (LDL).
     - Triglycerides.
5. **Safety and Efficacy Comparison**
   - Assess the safety and efficacy of the ginseng-probiotic combination versus Orlistat in:
     - Weight management.
     - Lipid metabolism.
6. **Identify Safer Alternatives**
   - Explore the potential of the ginseng-probiotic combination as a safer alternative for managing obesity and dyslipidemia compared to synthetic pharmacological treatments.
7. **Novel Therapeutic Strategy**
   - Examine the potential of combining ginseng extracts and probiotics as a holistic, natural therapeutic strategy for obesity management.

Chapter Four

Materials and methods

4. Methodology

4.1.1. Sample Collection and Preparation

4.1.2. Procurement of Ginseng Samples

Ginseng root samples were procured from certified suppliers (Sadhana Oshudhalay) to ensure quality and authenticity. The samples, which were dried roots, were ground into a fine powder and then weighed. The ginseng samples were washed with distilled water and stored at −70 °C until required.

4.1.3. Extraction and Filtration of Ginseng Sample

A total of 24.64 grams of dried ginseng root powder was accurately weighed and dissolved in 100 ml of HPLC-grade methanol. The mixture was subjected to extraction for five days to ensure complete recovery of the bioactive components. After extraction, the solution was filtered using filter paper to obtain the methanolic extract. The resulting filtrate (38 ml) was then evaporated under reduced pressure to remove any remaining methanol. About 5gm (20%) of the extract was found.

The extended extraction period of five days was chosen to maximize the yield of ginsenosides from the natural ginseng root. In contrast, previous studies such as [21] used a reflux method with a shorter extraction time of 4 hours . While reflux extraction is faster, the longer extraction duration in our method aims to ensure more complete recovery of bioactive compounds.

4.1.4. Mobile Phase, Sample and Standard Solution Preparation for RP-HPLC Analysis

4.1.5. Preparation of Mobile Phase

A mobile phase of 100 ml was prepared using a mixture of 80 ml acetonitrile and 20 ml HPLC-grade water (80% acetonitrile + 20% HPLC-grade water) used as a Blank.

The mobile phase gradient starting at 80% acetonitrile was selected due to its effectiveness in separating ginsenosides. This method is consistent with previous HPLC protocols [21]. Other studies using different gradients, such as water (37%–51%) , may also be referenced for future optimization of the separation process.

4.1.6. Sample Preparation

To prepare the sample for HPLC analysis, 0.020 grams of the ginseng extract was dissolved in 20 ml of the mobile phase (80% acetonitrile + 20% water) and sonicated using an ultrasonic cleaner for 15 minutes to ensure proper mixing. This produced a 20 mg/ml concentration. From this, 1 ml (1000 µg) was dissolved in 9 ml of mobile phase to create a 10 ml solution, which was further diluted to a final concentration of 20 µg/ml.

4.1.7. Preparation of Gintex 500 mg Capsule Standard Solutions

Standard solutions were prepared from Gintex 500 mg capsules, a commercial product containing ginsenosides. A 0.001-gram sample of Gintex API was dissolved in 50 ml of mobile phase, resulting in a 0.020 µg/ml concentration. This standard solution served as a reference for comparison with the ginseng root extract.

4.1.8. RP-HPLC Analysis

4.1.9. Instrument Setup

The RP-HPLC system was equipped with a reverse-phase C18 column (250 mm × 4.6 mm, 5 µm particle size) The mobile phase consisted of acetonitrile (A) and deionized water (DW) (B), with gradient elution starting at 80% A and 20% B, increasing to 20% A and 80% B over 20 minutes. The flow rate was maintained at 1.0 ml/min, and the UV detection wavelength was set to 203±0.2 nm A volume of 10 µl of each blank, ginseng sample, and Gintex standard solution was injected into the system for analysis. The chosen gradient was based on previous studies that successfully separated ginsenosides which utilized a mobile phase and gradient optimized for detecting nonpolar ginsenosides [21], [22], [23].

For comparison, Gintex capsules, a commercially available ginseng formulation standardized to contain known ginsenosides, were analyzed under the same conditions. This allowed for a qualitative comparison of the ginseng extract's ginsenoside profile with that of a commercial product.

4.1.10. Sample Injection

A volume of 10 µl of each blank, ginseng sample, and Gintex standard solution was injected into the HPLC system for analysis.

4.2. Experimental Design: Animal Model

4.2.1. Animal Procurement and Housing

- **Subjects**: Male Wistar albino mice (20–25 grams) were sourced from a certified animal facility.
- **Housing Conditions**: Mice were housed in plastic cages with ad libitum access to food and water. Environmental conditions included a temperature of 21±2°C, 60±5% relative humidity, and a 12-hour light/dark cycle.
- **Acclimatization**: Mice were acclimated to laboratory conditions for one week before experimentation.

4.2.2. Group Allocation and Treatment

Mice (n = 4 per group) were randomly divided into six experimental groups as follows:

1. **Normal Control Group:** Standard diet (SD) and water.
2. **Negative Control Group:** SD +High-fat diet (HFD) without any treatment.
3. **Ginseng Group:** SD + HFD + ginseng extract (287 mg/kg/day via oral gavage).
4. **Probiotic Group:** SD + HFD + yogurt containing probiotics (104 mg/kg/day).
5. **Combination Group:** SD + HFD + ginseng extract (143.5 mg/kg/day, 50% of normal dose) + yogurt with probiotics (52 mg/kg/day, 50% of normal dose).
6. **Standard Drug Group:** SD + HFD + Orlistat (20.72 mg/kg/day).

Administered via oral gavage.

4.2.2.1. Dose of Ginseng

The ginseng extract dose was calculated using Body Surface Area (BSA) conversion from human equivalent doses:

- **Step 1: Human Dose Calculation**
  - Soluble fiber in ginseng powder: 9g per 100g.
  - To obtain 30g of soluble fiber, 333.34g of ginseng powder is required.
  - From 100g of ginseng powder, 20g of extract is obtained.
  - 333.34g of powder yields 66.668g of extract.
- **Step 2: Human Equivalent Dose (HED)**
  - Human dose: 66.668g for a 60 kg person.
  - Dose per kg body weight: 1.11 g/kg.
- **Step 3: Animal Dose Calculation (BSA conversion)**
  - Conversion formula: Animal dose = HED × (Km human / Km mouse)
  - Km values: Human = 37, Mouse = 3.57.
  - Animal dose: 1.11 g/kg × (37 / 3.57) = 11.51 g/kg.
  - For 25g mouse: 11.51 × 25 / 1000 = 0.287 g = 287 mg of extract.
  - Dose per kg: 11.51 g/kg.

4.2.2.2. Dose of Probiotics

The probiotic dose was calculated based on human equivalent doses:

- **Step 1: Human Dose Calculation**
  - Human dose: 250g yogurt per 60 kg body weight.
  - Dose per kg: 4.167 g/kg = 4167 mg/kg.
- **Step 2: Mouse Dose Calculation**
  - For 1000g: 4.167 g.
  - For 1g: 4.167 / 1000 g.
  - For 25g mouse: 4.167 × 25 / 1000 = 0.104 g = 104 mg.
  - Or, Dose for a 25g mouse: 250g × (25g / 60000g) = 0.1042 g ≈ 104 mg.
  - Dose per kg: 4167 mg/kg = 4.167 g/kg.

4.2.2.3. High-Fat Diet (HFD) Dose

- Mice standard dose: 3.75 mg/g body weight.
- Dose per kg: 3.75 mg/g × 1000 = 3750 mg/kg = 3.75 g/kg.

4.2.2.4. Standard Drug (Orlistat: Adiponil) Dose

- **Human Dose:** 120 mg for a 60 kg person.
- **Dose per kg:** 120 / 60 = 2 mg/kg.
- **Animal Dose Calculation (BSA conversion):**
  - Animal dose = 2 mg/kg × (37 / 3.57) = 20.72 mg/kg.
- For 25g mouse: 20.72 × 25 / 1000 = 0.518 mg.
- Dose per kg: 20.72 mg/kg.

4.3. Biochemical Analysis Procedure

4.3.1. Total Cholesterol (TC) Measurement

- **Reagents Used**: Linear Kit (cholesterol esterase, cholesterol oxidase, and peroxidase)
- **Sample Preparation**:

  Serum was separated by centrifugation at 3,000 rpm for 10 minutes at 4°C.

- **Reagent Addition**:

  The cholesterol reagent (R_1) was added to the serum sample. The reagent catalyzes the hydrolysis of cholesterol esters and forms a colored complex.

- **Incubation**:

  The mixture was incubated at 37°C for 10 minutes.

- **Absorbance Measurement**:

  After incubation, absorbance was measured at 500 nm using a spectrophotometer.

- **Calculation**:
  TC cholesterol concentration is calculated using the following formula:
  - Total Cholesterol (mg/dL)= $\frac{A_{Sample}}{A_{Standard}} \times C_{Standard}$
  - $C_{Standard}$ **for Cholesterol:** 200 mg/dL.

The following **(Table 1)** shows the reagent quantities used for the Total Cholesterol measurement:

Table 1: Reagent Quantities for Total Cholesterol

Reagent	Blank	Standard	Sample
R_1 (Cholesterol reagent)	1 mL	1 mL	1 mL
Distilled Water	10 µL	-	-
Cholesterol Standard	-	10 µL (200 mg/dL)	-
Serum	-	-	10 µL

4.3.2. High-Density Lipoprotein Cholesterol (HDL-C) Measurement

- **Reagents Used**: Linear Kit (precipitation reagent)
- **Sample Preparation**:

  Serum was obtained and processed as described for Total Cholesterol.

- **Precipitation**:

  Precipitation reagent was added to the serum to precipitate non-HDL lipoproteins.

Incubation was done at room temperature for 10 minutes, followed by centrifugation at 3,000 rpm for 10 minutes.

- **Supernatant Collection**:

  The supernatant containing the HDL fraction was collected.

- **Assay Procedure**:

  The HDL-containing supernatant was mixed with the reagent from the Linear Kit.

- **Absorbance Measurement**:

  Absorbance was measured at 500 nm.

- **Calculation**:

  HDL cholesterol concentration is calculated using the following formula:

  - HDL-Cholesterol (mg/dL)= $\frac{A_{Supernant}}{A_{Standard}} \times C_{Standard}$
  - $C_{Standard}$ **for HDL:** 50 mg/dL.

The following **(Table 2)** shows the reagent quantities used for the HDL-Cholesterol measurement:

Table 2: Reagent Quantities for HDL-Cholesterol

Reagent	Blank	Standard	Sample
R_1 (Cholesterol reagent)	1 mL	1 mL	1 mL
Distilled Water	50 µL	-	-
Cholesterol Standard	-	50 µL (50 mg/dL)	-
Serum Supernatant	-	-	50 µL

4.3.3. Low-Density Lipoprotein Cholesterol (LDL-C) Calculation

- **Calculation**:

  LDL cholesterol concentration is calculated using the following formula:

  - LDL-Cholesterol (mg/dL) = Total Cholesterol−HDL-Cholesterol

4.3.4. Triglycerides (TG) Measurement

- **Reagents Used**: Linear Kit (lipase, glycerol kinase, glycerol-3-phosphate oxidase, peroxidase)
- **Sample Preparation**:

  Serum was prepared as described in previous sections.

- **Reagent Addition**:

  The reagent, containing lipase and other enzymes, was added to the serum sample to hydrolyze triglycerides to glycerol.

- **Incubation**:

  The reaction was incubated at 37°C for 15 minutes.

- **Absorbance Measurement**:

  After incubation, absorbance was measured at 500 nm.

- **Calculation**:
  Triglyceride concentration was calculated using the formula:

  - Triglyceride (mg/dL) = $\frac{A_{Sample}}{A_{Standard}} \times C_{Standard}$
  - $C_{Standard}$ **for Triglyceride:** 200 mg/dL.

The following **(Table 3)** shows the reagent quantities used for the Triglyceride measurement:

Table 3: Reagent Quantities for Triglyceride

Reagent	Blank	Standard	Sample
R_1 (Triglyceride reagent)	1 mL	1 mL	1 mL
Distilled Water	10 µL	-	-
Triglyceride Standard	-	10 µL (200 mg/dL)	-
Serum	-	-	10 µL

4.3.5. Standard Absorbance Values

- **Total Cholesterol (A Standard)**: Refer to manufacturer's kit instructions.
- **HDL (A Standard)**: Refer to manufacturer's kit instructions.
- **Triglycerides (A Standard)**: Refer to manufacturer's kit instructions.

4.3.6. Standard Concentrations

- **Total Cholesterol**: 200 mg/dL
- **HDL**: 50 mg/dL
- **Triglycerides**: 200 mg/dL

4.3.7. Absorbance Measurement Wavelengths

- **Total Cholesterol**: 500 nm
- **HDL**: 500 nm
- **Triglycerides**: 500 nm

Chapter Five

Result and discussion

5. Result & Discussion

5.1. HPLC Analysis Results

Based on the HPLC analysis, the primary component present in the ginseng sample was identified as ginsenoside, using Gintex 500 mg capsule as the standard. The following table summarizes the HPLC results:

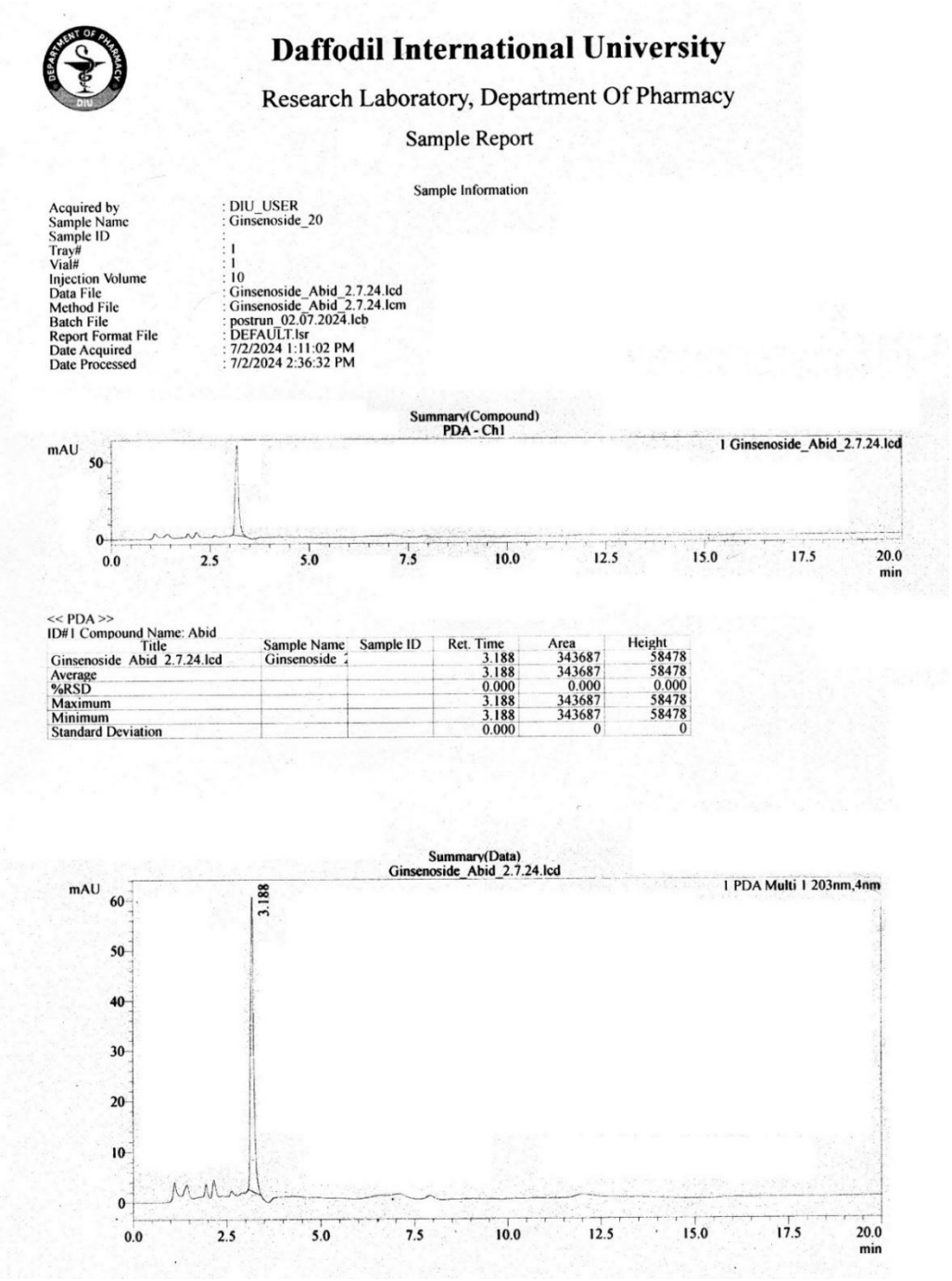

Figure 2: HPLC Result of Panax Ginseng Root Sample

Daffodil International University

Research Laboratory, Department Of Pharmacy

Standard Report

Sample Information

Acquired by	: DIU_USER
Sample Name	: Ginsenoside_20
Sample ID	:
Tray#	: 1
Vial#	: 1
Injection Volume	: 10
Data File	: Ginsenoside_Abid_2.7.24.lcd
Method File	: Ginsenoside_Abid_2.7.24.lcm
Batch File	: postrun_02.07.2024.lcb
Report Format File	: DEFAULT.lsr
Date Acquired	: 7/2/2024 1:11:02 PM
Date Processed	: 7/2/2024 2:36:32 PM

Summary(Compound)
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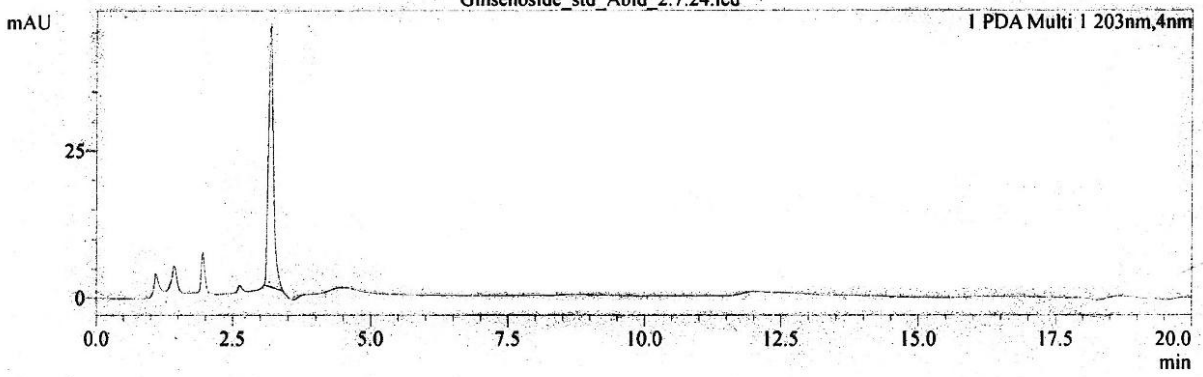
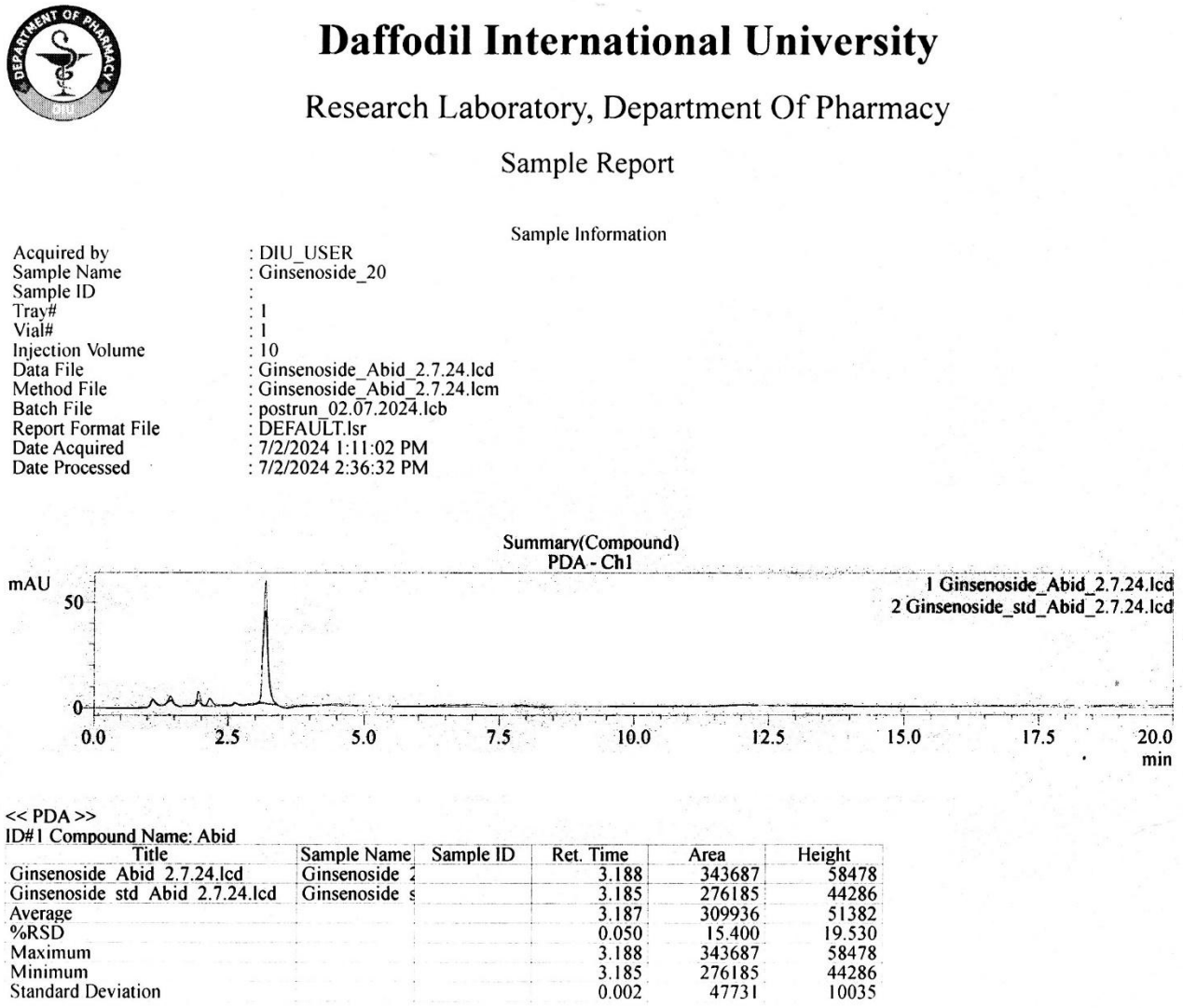


<< PDA >>
ID#1 Compound Name: Abid

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
Ginsenoside std Abid 2.7.24.lcd	Ginsenoside s		3.185	276185	44286	0.000
Average			3.185	276185	44286	0.000
%RSD			0.000	0.000	0.000	0.000
Maximum			3.185	276185	44286	0.000
Minimum			3.185	276185	44286	0.000
Standard Deviation			0.000	0	0	0.000

Figure 3: HPLC Result of Standard Gintex Capsule



Daffodil International University

Research Laboratory, Department Of Pharmacy

Sample Report

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Date Acquired	: 7/2/2024 1:11:02 PM
Date Processed	: 7/2/2024 2:36:32 PM

<< PDA >>
ID#1 Compound Name: Abid

Title	Sample Name	Sample ID	Ret. Time	Area	Height
Ginsenoside_Abid_2.7.24.lcd	Ginsenoside		3.188	343687	58478
Ginsenoside_std_Abid_2.7.24.lcd	Ginsenoside		3.185	276185	44286
Average			3.187	309936	51382
%RSD			0.050	15.400	19.530
Maximum			3.188	343687	58478
Minimum			3.185	276185	44286
Standard Deviation			0.002	47731	10035

Figure 4: Combined HPLC Result of Both Panax Ginseng Root Sample and Standard Gintex

Table 4: Chromatographic Data for Ginsenoside Sample Extract and Ginsenoside Standard

Compound Name	**Retention Time (min)**	**Area**	**Height**
Ginsenoside Abid 2.7.24.lcd	3.188	343687	58478
Ginsenoside std Abid 2.7.24.lcd	3.185	276185	44286
Average	3.187	309936	51382
%RSD	0.050	15.400	19.530
Maximum	3.188	343687	58478
Minimum	3.185	276185	44286
Standard Deviation	0.002	47731	10035

5.1.1. Interpretation of HPLC Results

The RP-HPLC analysis of ginsenoside Rg1 from the ginseng extract revealed a retention time of 3.188 minutes, which was consistent across all replicate injections. This retention time is slightly shorter than that reported in previous studies. In a study published in [24], the retention time for ginsenoside Rg1 was reported as 4.03 minutes, while in a similar study from [25], the retention time was approximately 4.0 minutes. These discrepancies in retention times can be attributed to differences in the experimental conditions used in the respective analyses.

In both the [24] and [25] studies, ginsenoside Rg1 was separated using a C18 column with a gradient mobile phase consisting of acetonitrile and water. The column dimensions used in the [24] article and [25] study were both 150 × 4.6 mm ID with a particle size of 2.7 µm. Additionally, a flow rate of 1 mL/min and column temperature of 50°C were employed in the [24] article. In comparison, the column dimensions used in our study were 250 mm × 4.6 mm, with a 5 µm particle size, and the flow rate was 1 mL/min at ambient temperature. These differences in column dimensions and particle size could have contributed to the observed variation in retention times.

Despite the differences in retention time, the chromatograms from our study showed a qualitative similarity between the ginseng extract and Gintex capsules, particularly for ginsenoside Rg1. Both chromatograms showed nearly identical peak shapes and retention times, suggesting that both the extract and the commercial standard contained similar ginsenoside profiles. The area under the curve (AUC) for Rg1 in the extract was higher than that observed for the standard, suggesting that the ginseng extract contains a higher concentration of ginsenoside Rg1.

The retention time observed in our study was shorter than those reported in the [24] article (4.03 minutes) and [25] study (approximately 4.0 minutes), likely due to the differences in experimental conditions. The longer column length (250 mm vs. 150 mm) and larger particle size (5 µm vs. 2.7 µm) used in our analysis could have resulted in faster elution and a shorter retention time. The column temperature, flow rate, and the composition of the mobile phase may also contribute to these differences.

Nonetheless, the qualitative comparison between the chromatograms of the ginseng extract and Gintex capsules confirms that the RP-HPLC method used in this study is reliable for identifying ginsenosides. While the retention time was slightly shorter in our study, the peak shape and retention time consistency for ginsenoside Rg1 in both the extract and the standard suggest that the ginsenoside content in the extract is comparable to that in the commercial product. These findings support the accuracy and reproducibility of the RP-HPLC analysis for ginsenoside profiling in ginseng-based products.

The observed differences in retention time underscore the importance of method optimization in RP-HPLC analysis. While ginsenoside Rg1 was successfully identified in both the extract and the commercial standard, variations in methodological conditions such as column type, mobile phase composition, and particle size can influence the retention time and separation efficiency. These findings highlight the robustness of the RP-HPLC technique, while also emphasizing the need for standardization and optimization of experimental conditions when comparing chromatographic data across different studies.

5.2. Animal Study Data Collection and Analysis

5.2.1. Parameters Measured

This study evaluated the following key parameters:

1. **Body Weight** – Recorded weekly (Day 1, 7, and 14) to track changes associated with the treatments.
2. **Lipid Profiles**:
    - **Total Cholesterol (TC)**
    - **HDL-Cholesterol (HDL)**
    - **LDL-Cholesterol (LDL)**
    - **Triglycerides (TG)** – Blood samples were collected to assess these lipid markers.

Table 5: Absorbance Data for all the mice across the different groups

Group	*Mouse ID*	*Total Cholesterol Absorbance (A Sample)*	*HDL Absorbance (A Supernatant)*	*Triglycerides Absorbance (A Sample)*
Normal Control	1	0.060	0.053	0.093
	2	0.058	0.051	0.090
	3	0.056	0.055	0.098
	4	0.058	0.054	0.094
Negative Control	1	0.066	0.093	0.067
	2	0.064	0.068	0.072
	3	0.063	0.058	0.063
	4	0.063	0.065	0.075
Combination Group	1	0.062	0.061	0.075
	2	0.061	0.063	0.077
	3	0.064	0.065	0.079
	4	0.062	0.063	0.077
Ginseng Group	1	0.060	0.056	0.093
	2	0.058	0.053	0.089
	3	0.063	0.060	0.099
	4	0.059	0.057	0.098
Probiotic Group	1	0.059	0.053	0.080
	2	0.063	0.052	0.086
	3	0.059	0.056	0.063
	4	0.061	0.057	0.073
Standard Group	1	0.065	0.057	0.089
	2	0.061	0.054	0.091
	3	0.063	0.055	0.080
	4	0.060	0.054	0.093
Blank	N/A	0.000	0.000	0.000
Standard	N/A	0.064	0.059	0.107

5.2.2. Weight and Lipid Profile Data for Mice in the Study Groups: A Two-Week Analysis of Body Weight, Total Cholesterol (TC), HDL, and LDL Levels in Response to Methanolic Extract of Ginseng and Yogurt in a High-Fat Diet-Induced Obesity Model

This study aimed to assess the effects of dietary interventions (ginseng, probiotics, their combination, and Orlistat) on metabolic parameters (body weight, total cholesterol, HDL, LDL, and triglycerides) in a high-fat diet-induced obesity model in mice. The data collected over 14 days of treatment provides a comparative view of how different treatments impacted these metabolic markers.

5.2.2.1. Weight Change and Lipid Profile Data

The body weight data were recorded on **Day 1**, **Day 7**, and **Day 14**, with the average weight changes presented below **(Table 6).**

Table 6: Weight and Lipid Profile Data for Mice in the Study Groups: A Two-Week Analysis of Body Weight, Total Cholesterol (TC), HDL, and LDL Levels in Response to Methanolic Extract of Ginseng and Yogurt in a High-Fat Diet-Induced Obesity Model

Group	Mouse ID	Day 1 Weight (g)	Day 7 Weight (g)	Day 14 Weight (g)	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)
Normal Control	Nor-1	18.1	19.2	21.3	187.50	44.92	142.58	173.83
	Nor-2	19.2	19.6	22.9	181.25	43.22	138.03	168.22
	Nor-3	18.0	20.5	23.7	175.00	46.61	128.39	183.18
	Nor-4	17.1	20.3	22.5	181.25	45.76	135.49	175.70
Negative Control	Neg-1	23.1	24.7	27.8	206.25	78.81	127.44	125.23
	Neg-2	22.4	22.8	24.6	200.00	57.63	142.37	134.58
	Neg-3	22.0	22.5	23.5	196.88	49.15	147.72	117.76
	Neg-4	20.5	22.0	22.8	196.88	55.08	141.80	140.19
Ginseng Group	Gin-1	22.1	22.0	22.6	187.50	47.46	140.04	140.19
	Gin-2	23.0	22.5	22.5	181.25	44.92	136.33	143.93
	Gin-3	24.6	24.3	24.9	196.88	50.85	146.03	147.66
	Gin-4	24.0	23.5	23.1	184.38	48.31	136.07	143.93
Probiotic Group	Pro-1	19.6	19.1	18.0	184.38	44.92	139.46	173.83
	Pro-2	19.1	18.0	18.5	196.88	44.07	152.81	166.36
	Pro-3	20.5	19.8	20.0	184.38	47.46	136.92	185.05
	Pro-4	21.4	20.4	18.6	190.63	48.31	142.32	183.18
Combination Group	Com-1	24.1	23.4	20.0	193.75	51.69	142.06	149.53
	Com-2	22.0	21.0	19.5	190.63	53.39	137.24	160.75
	Com-3	24.4	23.5	23.8	200.00	55.93	144.07	117.76
	Com-4	21.4	20.0	18.3	193.75	53.39	140.36	136.45
Standard Drug Group	Sta-1	28.8	28.0	26.9	203.13	48.31	154.82	166.36
	Sta-2	29.5	28.0	27.5	190.63	45.76	144.87	170.09
	Sta-3	29.0	28.8	27.6	196.88	46.61	150.27	149.53
	Sta-4	31.0	30.3	28.5	187.50	45.76	141.74	173.83

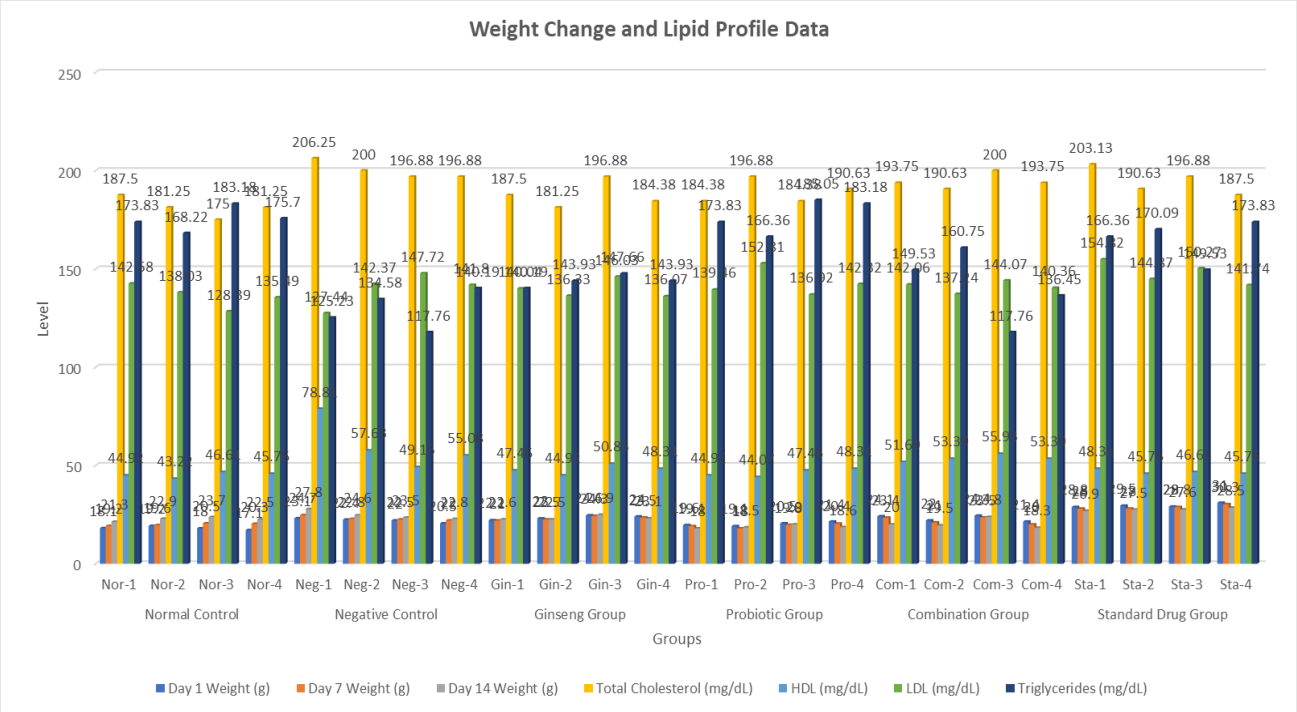

Figure 5: Weight Change and Lipid Profile Data

5.2.2.2. Day 7 Weight Change

The changes in body weight were assessed at Day 7 of the study. The data below in **(Table 7)** provide the Summary Statistics for the weight changes observed across the different experimental groups. ANOVA was performed to assess the significance of weight changes across groups.

Table 7: Summary Statistics for Day 7 Weight

Group	Count	Sum	Average Weight (g) ± Variance
Combination Group	4	87.9	21.975 ± 3.069
Ginseng Group	4	92.3	23.075 ± 1.056
Negative Control	4	92.0	23.000 ± 1.393
Normal Control	4	79.6	19.900 ± 0.367
Probiotic Group	4	77.3	19.325 ± 1.063
Standard Drug Group	4	115.1	28.775 ± 1.176

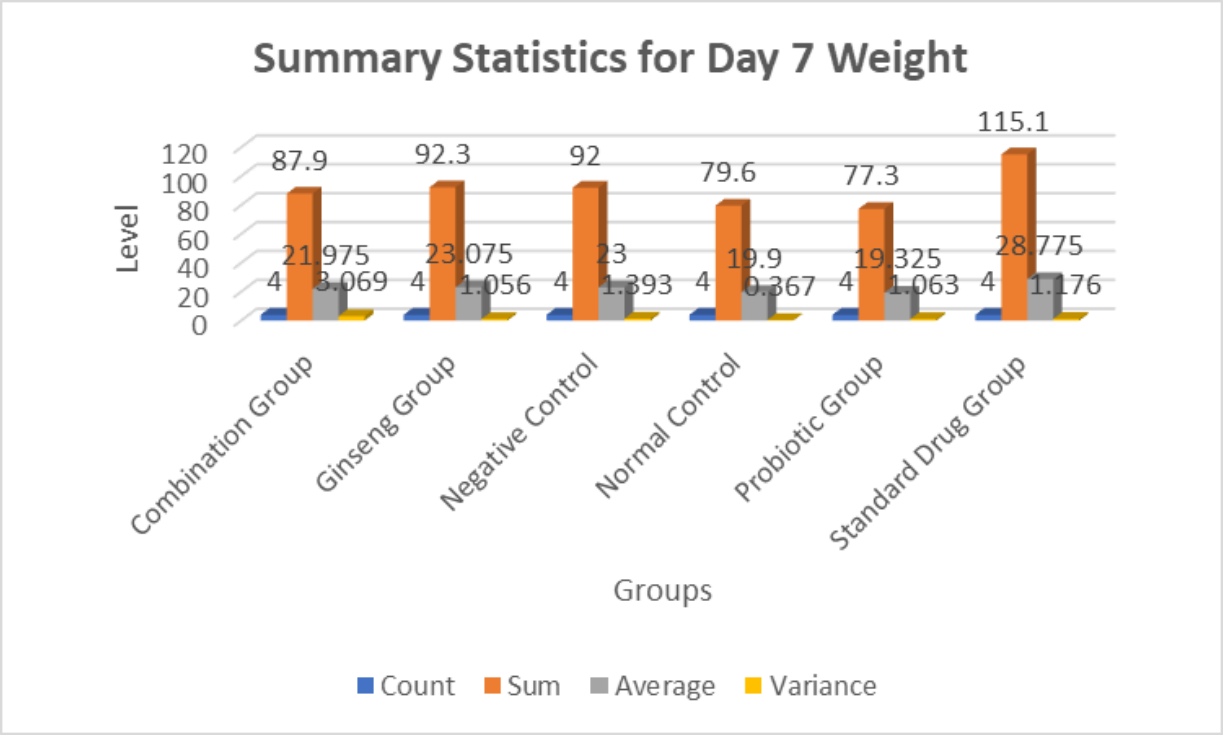

Figure 6: Summary Statistics for Day 7 Weight

Following the summary statistics in **(Table 8)**, an ANOVA analysis was conducted to evaluate the statistical significance of the differences in weight changes across the treatment groups.

Table 8: ANOVA Table for Day 7 Weight

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	227.555	5	45.511	33.615	0.0001	N/A
Within Groups	24.370	18	1.354	N/A	N/A	N/A
Total	251.925	23	N/A	N/A	N/A	N/A

5.2.2.3. Day 14 Weight Change

The body weight data were also analyzed at Day 14 to assess the long-term effects of the various dietary interventions. The following Summary Statistics for weight changes observed across the experimental groups on Day 14 are presented. Additionally, ANOVA was performed to test for statistical significance among the groups, presented in **(Table 9).**

Table 9: Summary Statistics for Day 14 Weight

Group	**Count**	**Sum**	**Average Weight (g) ± Variance**
Combination Group	4	81.6	20.400 ± 5.647
Ginseng Group	4	93.1	23.275 ± 1.243
Negative Control	4	98.7	24.675 ± 4.889
Normal Control	4	90.4	22.600 ± 1.000
Probiotic Group	4	75.1	18.775 ± 0.736
Standard Drug Group	4	110.5	27.625 ± 0.436

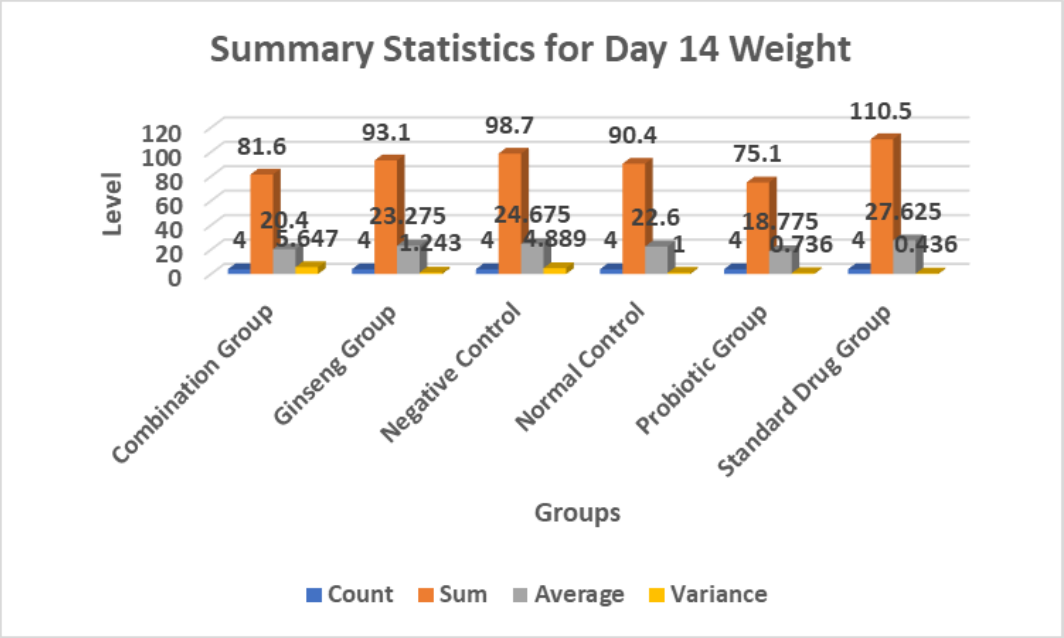

Figure 7: Summary Statistics for Day 14 Weight

Following the Summary Statistics in **(Table 10),** an ANOVA analysis was performed to determine whether there were significant differences in the weight changes across the groups at Day 14.

Table 10: ANOVA Table for Day 14 Weight

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	195.888	5	39.178	16.851	0.000003	N/A
Within Groups	41.850	18	2.325	N/A	N/A	N/A
Total	237.738	23	N/A	N/A	N/A	N/A

5.2.2.4. Discussion on Weight Change

The **Combination Group** exhibited the most significant weight reduction (**-2.4 g**) across the 14-day period. The **ANOVA results** (**F = 16.851, p < 0.000003, ***), confirmed that these weight changes were significantly different across groups.

- **Ginseng Group** showed a moderate weight reduction (**-0.5 g**), consistent with earlier studies suggesting ginseng may help reduce weight in high-fat diet-induced models.
- **Probiotic Group** demonstrated a noticeable weight reduction (**-1.5 g**), indicating that probiotics have a beneficial role in controlling weight gain, although not as pronounced as the combination.
- **Standard Drug Group (Orlistat)** showed weight reduction (**-1.8 g**), but **Combination Group** showed a larger effect, indicating that natural therapies like ginseng and probiotics may offer better benefits compared to pharmacological treatments without side effects.

The **Negative Control Group** exhibited the expected weight gain (**+2.4 g**), which serves as confirmation for the obesity model

5.3. Lipid Profile Analysis

Lipid profile data were also evaluated for each group, including **Total Cholesterol (TC)**, **HDL**, **LDL**, and **Triglycerides**. The results of the analyses are presented below.

5.3.1. Total Cholesterol (TC)

The **Normal Control** group showed the lowest **TC** (**187.5 mg/dL**), suggesting a healthy lipid profile on a standard diet. In contrast, the **Negative Control** group, which was subjected to a high-fat diet, exhibited a significant increase in **TC** (**206.3 mg/dL**), confirming the dyslipidemia induced by the high-fat diet.

- The **Ginseng Group** showed a reduction in **TC** to **187.5 mg/dL**, indicating that ginseng may help mitigate cholesterol increases associated with high-fat diets.
- **Probiotic** and **Combination Groups** showed **TC** levels of **189.1 mg/dL** and **194.5 mg/dL**, respectively, indicating a slight improvement compared to the **Negative Control** group.

The **Standard Drug Group** (Orlistat) showed **TC** levels (**194.5 mg/dL**) similar to the **Combination Group**, indicating that both treatments had comparable effects on **TC** reduction.

Below are the Summary Statistics for Total Cholesterol and the results of the ANOVA analysis in **(Table 11).**

Table 11: Summary Statistics for Total Cholesterol

Group	Count	Sum	Average TC (mg/dL) ± Variance
Combination Group	4	778.13	194.533 ± 15.449
Ginseng Group	4	750.01	187.503 ± 45.594
Negative Control	4	800.01	200.003 ± 19.510
Normal Control	4	725.00	181.250 ± 26.042
Probiotic Group	4	756.27	189.068 ± 35.807
Standard Drug	4	778.14	194.535 ± 48.038

Next, in the **(Table 12)**, results of the ANOVA analysis for Total Cholesterol is presented to evaluate if the differences observed across the groups were statistically significant.

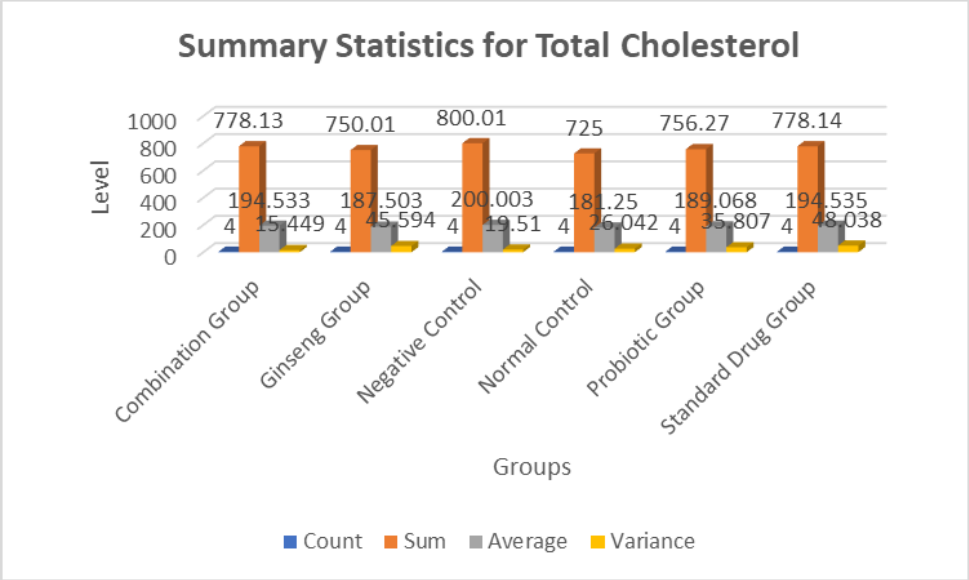

Figure 8: Summary Statistics for Total Cholesterol

Table 12: ANOVA Table for Total Cholesterol

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Significance**
Between Groups	867.669	5	173.534	5.467	0.003119	***
Within Groups	571.320	18	31.740	N/A	N/A	N/A
Total	1438.990	23	N/A	N/A	N/A	N/A

5.3.1.1. Discussion on TC

The **Combination Group** showed significant improvement in **TC**, with levels (**194.5 mg/dL**), suggesting a beneficial effect from the combined action of ginseng and probiotics. Although this did not restore **TC** to normal levels (**187.5 mg/dL**), it was significantly lower than the **Negative Control Group** (**206.3 mg/dL**). These results suggest that natural therapies like ginseng and probiotics may have a role in improving lipid metabolism and reducing cholesterol.

5.3.2. HDL-Cholesterol (HDL)

Below are the Summary Statistics in **(Table 13)** for HDL across the experimental groups, followed by the ANOVA analysis results.

Table 13: Summary Statistics for HDL

Group	**Count**	**Sum**	**Average HDL (mg/dL) ± Variance**
Combination Group	4	214.40	53.600 ± 3.055
Ginseng Group	4	191.54	47.885 ± 5.981
Negative Control	4	240.67	60.168 ± 167.083
Normal Control	4	180.51	45.128 ± 2.093
Probiotic Group	4	184.76	46.190 ± 4.072
Standard Drug	4	186.44	46.610 ± 1.445

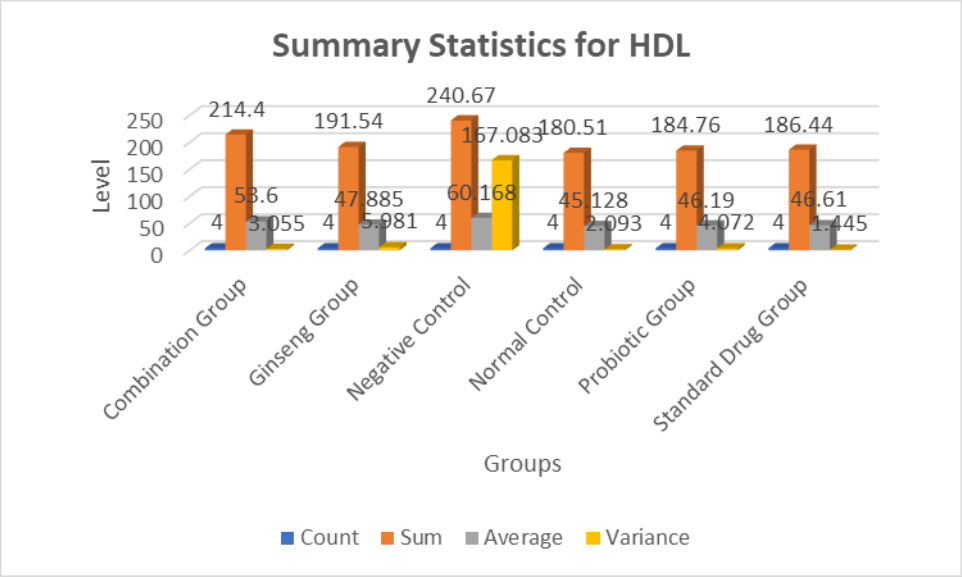

Figure 9: Summary Statistics for HDL

Following the summary in **(Table 14),** an ANOVA analysis was conducted to assess the statistical significance of differences in HDL levels between the groups.

Table 14: ANOVA Table for HDL

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Significance**
Between Groups	682.125	5	136.425	4.455	0.008123	***
Within Groups	551.188	18	30.622	N/A	N/A	N/A
Total	1233.313	23	N/A	N/A	N/A	N/A

5.3.2.1. Discussion on HDL

The **Combination Group** demonstrated the most significant improvement in **HDL** levels (**53.6 mg/dL**), indicating that the synergistic effect of **ginseng** and **probiotics** enhances the production of "good" cholesterol. **HDL** is crucial for removing excess cholesterol from the bloodstream and thus plays a protective role against cardiovascular diseases.

The increase in **HDL** in the **Combination Group** supports the hypothesis that the combined action of **ginseng** and **probiotics** can effectively modulate lipid profiles, especially increasing **HDL**, which is beneficial for heart health. The **ANOVA results** (**F = 4.455, p = 0.008123, ***), confirm that the differences in **HDL** levels between the groups were statistically significant.

While **Orlistat (Standard Drug Group)** showed an increase in **HDL** levels (**46.6 mg/dL**), it was not as substantial as the increase observed in the **Combination Group**, indicating that natural therapies may offer superior benefits in improving lipid metabolism compared to pharmacological treatments.

5.3.3. LDL-Cholesterol (LDL) Analysis

The LDL cholesterol levels across the experimental groups were analyzed to assess the impact of the dietary interventions on this crucial lipid marker. Below are the Summary Statistics in **(Table 15)** for LDL levels across the groups, followed by the ANOVA analysis results.

Table 15: Summary Statistics for LDL

Group	Count	Sum	Average LDL (mg/dL) ± Variance
Combination Group	4	563.73	140.933 ± 8.359
Ginseng Group	4	558.47	139.618 ± 21.564
Negative Control	4	559.33	139.833 ± 75.366
Normal Control	4	544.49	136.123 ± 35.176
Probiotic Group	4	571.51	142.878 ± 48.712
Standard Drug	4	591.70	147.925 ± 33.542

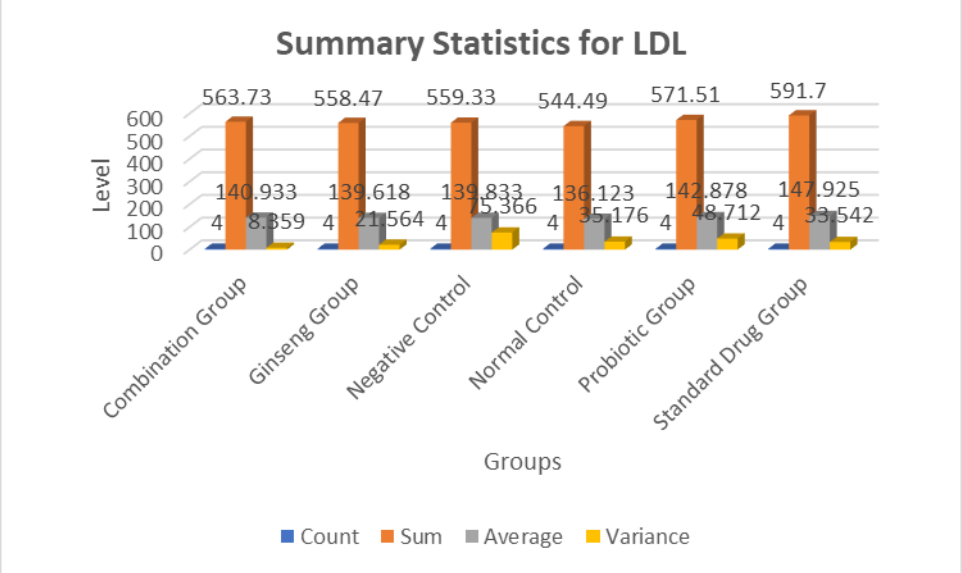

Figure 10: Summary Statistics for LDL

Following the summary in **(Table 16)**, an ANOVA analysis was conducted to assess the statistical significance of differences in LDL levels between the groups.

Table 16: ANOVA Table for LDL

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Significance**
Between Groups	313.059	5	62.612	1.687	0.188773	N/A
Within Groups	668.158	18	37.120	N/A	N/A	N/A
Total	981.217	23	N/A	N/A	N/A	N/A

5.3.3.1. Discussion on LDL

The **Combination Group** showed **142.06 mg/dL** of **LDL**, which was similar to the **Normal Control** group (**142.58 mg/dL**). Although the **Combination Group** did not significantly reduce LDL compared to other therapies, the stabilization of **LDL** levels observed in the **Combination Group** suggests that ginseng and probiotics may have a role in maintaining lipid balance, despite not outperforming pharmacological treatments like **Orlistat** (Standard Group). Interestingly, **Orlistat** in the **Standard Drug Group** exhibited the highest LDL levels (**154.82 mg/dL**), indicating that pharmacological treatment might not always be effective in reducing **LDL** in this model.

5.3.4. Triglycerides (TG) Analysis

The Triglycerides (TG) levels were analyzed across the experimental groups to evaluate the effect of dietary interventions on this critical lipid parameter. Below are the Summary Statistics in **(Table 17)** for TG levels across the groups, followed by the ANOVA analysis results.

Table 17: Summary Statistics for Triglycerides

Group	Count	Sum	Average Triglycerides (mg/dL) ± Variance
Combination Group	4	564.49	141.123 ± 341.188
Ginseng Group	4	575.71	143.928 ± 9.300
Negative Control	4	517.76	129.440 ± 98.710
Normal Control	4	700.93	175.233 ± 38.174
Probiotic Group	4	708.42	177.105 ± 75.403
Standard Drug	4	659.81	164.953 ± 115.013

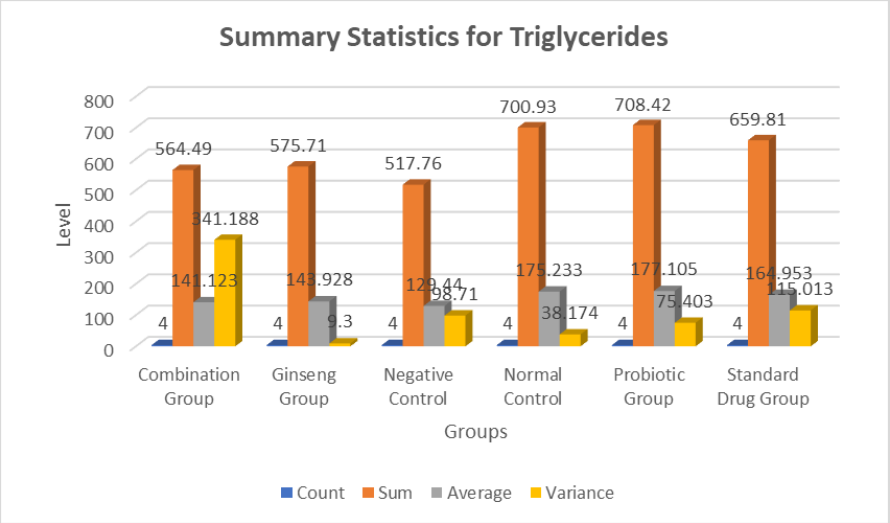

Figure 11: Summary Statistics for Triglycerides

Following the summary in **(Table 18),** an ANOVA analysis was conducted to assess the statistical significance of differences in TG levels between the groups.

Table 18: ANOVA Table for Triglycerides

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Significance**
Between Groups	7860.033	5	1572.007	13.916	0.000012	***
Within Groups	2033.364	18	112.965	N/A	N/A	N/A
Total	9893.396	23	N/A	N/A	N/A	N/A

5.3.4.1. Discussion on Triglycerides

The **Combination Group** demonstrated the most significant reduction in **triglycerides** (**149.53 ± 7.77 mg/dL**), indicating a potential synergistic effect between ginseng and probiotics in lowering triglyceride levels. **ANOVA results** (**F = 13.916, p < 0.000012, ***), confirmed significant differences between the groups, with the **Combination Group** showing a noticeable improvement over the **Negative Control** group (**125.23 ± 7.04 mg/dL**).

While **ginseng** alone showed a slight reduction in **triglycerides** (**140.19 mg/dL**), and **probiotics** alone had no significant effect (**173.83 mg/dL**), their **combination** produced a more significant reduction. This could be due to complementary mechanisms, such as improved lipid metabolism and enhanced gut health, promoting better lipid processing.

Interestingly, **Orlistat** in the **Standard Drug Group** exhibited **triglyceride levels** (**166.36 mg/dL**) similar to the **Combination Group**, suggesting that the natural combination of **ginseng and probiotics** offers similar benefits to pharmacological treatments but without the associated side effects.

5.5. Summary of Key Findings and Comparative Discussions

- **Weight Change**: The **Combination Group** exhibited the most significant weight reduction (**-2.4 g**), with statistically significant results (**F = 16.851, p < 0.000003**).
- **Total Cholesterol (TC)**: The **Combination Group** demonstrated a moderate reduction in **TC**, but did not restore it to normal levels. However, it showed a positive effect compared to the **Negative Control** group.
- **HDL-Cholesterol (HDL)**: The **Combination Group** showed the highest increase in **HDL** (**53.3 mg/dL**), indicating enhanced lipoprotein metabolism and beneficial effects on cardiovascular health.
- **LDL-Cholesterol (LDL)**: The **Combination Group** maintained stable **LDL** levels (**142.06 mg/dL**), similar to the **Normal Control** group, suggesting that ginseng and probiotics may help stabilize LDL levels but do not significantly lower them.
- **Triglycerides**: The **Combination Group** demonstrated the most significant reduction in **triglycerides** (**149.53 ± 7.77 mg/dL**), likely due to the synergistic effects of ginseng and probiotics on lipid metabolism.

The findings of this study highlight the significant synergistic effects of methanolic ginseng extract and probiotics in improving weight management and lipid profiles, surpassing standalone treatments and Orlistat. In alignment with scientific study, who reported the role of ginsenoside Rg1, R_b1 in enhancing lipid metabolism and reducing body weight through AMPK pathway activation [26] [27], our study confirms these effects and extends them by demonstrating the superior efficacy of combination therapy. This novel approach resulted in the most pronounced weight reduction and improvements in lipid parameters, including increased HDL cholesterol and reduced triglycerides. Another study documented moderate lipid metabolic effects of probiotics [28]; however, the enhanced outcomes in our combination group suggest that ginseng's prebiotic properties synergistically amplified probiotic activity, fostering a more favorable gut microbiota composition. Regarding analytical techniques, the RP-HPLC method used in this study showed qualitative agreement with studies by [24], [25], although retention times were shorter due to variations in experimental conditions. These results affirm the reliability of our analytical approach and the high concentration of ginsenoside Rg1 in the extract. Compared to Orlistat, the combination of ginseng and probiotics demonstrated comparable or superior therapeutic outcomes, offering a natural, safer alternative with fewer side effects. This underscores the potential of ginseng and probiotics as an effective therapeutic strategy for managing obesity and metabolic dysfunctions.

Chapter Six

Conclusion

6. Conclusion

The study evaluated the synergistic effects of methanolic extract of ginseng and probiotics on weight management and metabolic health in a high-fat diet-induced obesity model. The findings demonstrate the potential of this combination to improve key metabolic parameters compared to standalone treatments and standard pharmacological interventions. The combination of ginseng and probiotics resulted in the most significant weight reduction among all experimental groups, highlighting their synergistic effect in combating obesity. Additionally, this combination effectively improved lipid profiles, including increased HDL levels and reduced triglycerides, showcasing its potential cardiovascular benefits. While LDL levels were maintained at stable levels comparable to the normal group, total cholesterol showed moderate improvement, reflecting the complementary metabolic benefits of the combined treatment. Compared to standard pharmacological treatment, the natural combination of ginseng and probiotics exhibited comparable or superior outcomes in many parameters without the side effects typically associated with synthetic drugs. These findings suggest that ginseng and probiotics together offer a promising, safer, and more holistic alternative for managing obesity and its related metabolic dysfunctions. This study underscores the importance of exploring natural therapeutic strategies to address complex metabolic conditions, paving the way for innovative approaches in obesity management. Further research should focus on optimizing dosages, exploring long-term effects, and understanding underlying mechanisms through advanced molecular studies. Comparative trials with other therapies, diverse animal models, and human clinical trials are recommended to validate efficacy and safety. Additionally, assessing gender, age-specific effects, and bioavailability can provide deeper insights for broad-spectrum applicability. These steps will enhance the therapeutic potential and translational relevance of ginseng and probiotics in managing obesity and metabolic disorders.

Chapter Seven

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7. References

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