REPUBLIC OF TURKİYE
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

THE EFFECTS OF BROMELAIN ENZYME ADDITION TO HIGH LIPID AND HIGH PROTEIN DIETS ON NUTRITIONAL PHYSIOLOGY, TISSUE HISTOLOGY AND GENE EXPRESSION IN RATS

Prepared By
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Supervisor
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Ph.D. Thesis

September 2025
KAYSERİ

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SCIENTIFIC ETHICS SUITABILITY

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Omar Salah Ahmed Ahmed

The Ph.D. thesis entitled "**The Effects of Bromelain Enzyme Addition to High Lipid and High Protein Diets on Nutritional Physiology, Tissue Histology and Gene Expression in Rats**" has been prepared in accordance with Erciyes University Graduate School of Natural and Applied Sciences Thesis Preparation and Writing Guide.

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Omar Salah Ahmed Ahmed

September 2025, Kayseri

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Erciyes University, Graduate School of Natural and Applied Sciences
PhD Thesis, September 2025
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ABSTRACT

The study investigated the effects of bromelain addition to high-fat and high-protein diets on physiological and molecular in laboratory rats. In the study, a total of 25 laboratory rats (3-5 weeks old, weighing 200-220 grams) were allocated 5 treatment groups. The treatment groups as follows: T1: Control standard diet, T2: Standard diet + high-fat food (ghee) 4 g divided into 2 doses, morning and evening, T3: Standard diet + high protein food (albumin) 4 g divided into 2 doses morning and evening, T4: Standard diet + high-fat foods + bromelain enzyme 4 mg divided into two doses morning and evening and T5: Standard diet + high-protein foods + bromelain enzyme 4 mg divided into two doses morning and evening. Feeding rats with an additional 4 grams of fat and protein daily showed a highly significant body weight gain, adipose tissue accumulation, elevated liver enzymes, impaired kidney function, inflammatory responses (increased blood and white blood cell counts), platelet dysfunction, and increased oxidative stress. Increased non-alcoholic fatty liver disease (NAFLD), decreased glutathione peroxidase (GPx) activity, and increased malondialdehyde (MDA), gene expression of digestive enzymes (lipase, pepsin, trypsin). Bromelain addition (4 mg/day) effectively mitigated these adverse effects, improved kidney and liver function. At the molecular level, bromelain significantly increased lipase expression (427 fold compared to protein-fed rats), indicating improved fat digestion, while reducing the expression of pepsin (0.18 fold) and trypsin (0.44 fold) compared to protein-fed rats. In conclusion, bromelain has a potential therapeutic role in regulating digestion, oxidative stress, and organ health, thereby preventing metabolic, hepatic, renal, and molecular disorders resulting from diets high in fat and protein. This highlights its potential therapeutic role in regulating digestion, oxidative stress, and organ health.

Keywords: High protein, High lipid, Enzymes, Bromelain, Diets, Gene expression.

YÜKSEK LIPIDLI VE YÜKSEK PROTEINLI DIYETLERE BROMELAIN ENZIMININ EKLENMESININ SIÇANLARDA BESLENME FIZYOLOJISI, DOKU HISTOLOJISI VE GEN EKSPRESYONU ÜZERINDEKI ETKILERI

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ÖZET

Çalışmada, laboratuvar sıçanlarında yüksek yağlı ve yüksek proteinli diyete bromelain eklenmesinin fizyolojik ve moleküler etkileri araştırıldı. Çalışmada toplam 25 laboratuvar sıçanı (3-5 haftalık, 200-220 gram ağırlığında) 5 tedavi grubuna ayrıldı. Tedavi grupları şu şekildedir: T1: Kontrol standart diyet, T2: Standart diyet + yüksek yağlı besin (ghee) 4 gr, sabah ve akşam 2 doza bölünmüş, T3: Standart diyet + yüksek proteinli besin (albumin) 4 gr, sabah ve akşam 2 doza bölünmüş, T4: Standart diyet + yüksek yağlı besinler + bromelain enzimi 4 mg, sabah ve akşam 2 doza bölünmüş ve T5: Standart diyet + yüksek proteinli besinler + bromelain enzimi 4 mg, sabah ve akşam 2 doza bölünmüş. Sıçanlara günlük ilave 4 gram yağ ve protein verilmesi, son derece önemli vücut ağırlığı artışı, yağ dokusu birikimi, yükselmiş karaciğer enzimleri, bozulmuş böbrek fonksiyonu, inflamatuar yanıtlar (artmış kan ve beyaz kan hücresi sayıları), trombosit disfonksiyonu ve artmış oksidatif stres gösterdi. Alkolik olmayan yağlı karaciğer hastalığında (NAFLD) artış, glutatyon peroksidaz (GPx) aktivitesinde azalma ve malondialdehit (MDA) ile sindirim enzimlerinin (lipaz, pepsin, tripsin) gen ekspresyonunda artış görüldü. Bromelain ilavesi (4 mg/gün) bu olumsuz etkileri etkili bir şekilde hafifletti, böbrek ve karaciğer fonksiyonlarını iyileştirdi. Moleküler düzeyde, bromelain lipaz ekspresyonunu önemli ölçüde artırdı (proteinle beslenen sıçanlara kıyasla 427 kat), bu da yağ sindiriminin iyileştiğini gösterirken, proteinle beslenen sıçanlara kıyasla pepsin (0,18 kat) ve tripsin (0,44 kat) ekspresyonunu azalttı. Sonuç olarak, bromelain sindirimi, oksidatif stresi ve organ sağlığını düzenlemede potansiyel bir terapötik role sahiptir ve böylece yağ ve protein açısından zengin beslenmeden kaynaklanan metabolik, hepatik, renal ve moleküler bozuklukları önler. Bu durum, sindirimi, oksidatif stresi ve organ sağlığını düzenlemedeki potansiyel terapötik rolünü vurgular.

Anahtar Kelimeler: Yüksek protein, Yüksek lipid, Enzimler, Bromelain, Diyetler, Gen ifadesi.

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INTRODUCTION

Nutrition is key to maintaining human health and protecting against chronic diseases. The elements in food directly affect metabolism, organ function, and the quality and health of living cells (Chen et al., 2018). Excessive fats and proteins are the primary cause of obesity, metabolic disorders, gastrointestinal complications, weakened immunity, and decreased pancreatic enzyme production, exacerbating long-term health risks, obesity and related metabolic disorders, including kidney and liver dysfunction, are strongly linked to unhealthy dietary patterns rich in fats and proteins. A balanced healthy diet containing the macro and micronutrients our body needs will positively impact growth, enhance the health of vital tissues, increase immunity, and protect against external influences (Gombart et al., 2020). However, over consumption of high-fat and high-protein foods for long periods can cause metabolic, hormonal, and genetic problems, which negatively impacts overall health, especially obesity, high blood pressure, diabetes, kidney failure, liver damage, oxidative stress, decreased immunity, increased inflammation, cancerous diseases, in addition to metabolic and gene expression disorders and digestive system problems (Reynés and Palou, 2025). High-fat diets, in particular, are associated with fat accumulation, hyperlipidemia, increased production of reactive oxygen species (ROS), and excess free radicals that disrupt the function of mitochondria and damage cell membranes (Vial et al., 2011; Yang et al., 2006). Excessively high-protein diets can also contribute to renal overload, amino acid metabolism imbalance, and increased nitrogenous waste (Moundras et al., 1993). These factors can exacerbate oxidative stress and inflammation, leading to tissue integrity degradation, impaired physiological functions, and predisposing individuals to metabolic syndromes (Masenga et al., 2023). At the molecular level, oxidative damage to nucleic acids and proteins interferes with the regulation of gene expression, leading to aberrant signaling pathways that accelerate disease progression (Sung et al., 2013). Enzymes are a stimulant factor that play a vital role in regulating metabolism and

digestion after eating meals rich in fats and proteins, thus maintaining cell health (Bagchi and Ohia, 2021). Bromelain is one of the a natural agents for combating oxidative damage in the body. It is extracted from pineapple fruit a powerful antioxidant, antimicrobial growth, and anticoagulant, due to its ability to reduce fibrin levels (Varilla et al., 2021). Previous studies have confirmed bromelain's ability to improve nutrient absorption, enhance the body's ability to digest proteins, and reduce the production of reactive oxygen species (ROS). Furthermore, it can affect oxidation and reduction reactions within cells, helping to prevent DNA damage and combat genetic mutations. Bromelain can also regulate the gene expression of digestive enzymes, particularly trypsin and pepsin, by regulating the feedback mechanism that occurs between cell nuclei and receptors on their surfaces, the widespread prevalence of unhealthy foods in societies has contributed to the increased prevalence of diseases and metabolic disorders (Kiersnowska and Jakubczyk 2022; Kansakar et al. 2024).

Therefore, the study focused on clarifying the role of the bromelain enzyme administration in laboratory rats fed a high-fat and high-protein diet through nutritional physiology, blood parameters examination, tissue studies, and gene expression patterns in order to provide comprehensive insights into the nutritional and therapeutic functions of bromelain.

CHAPTER 1

LITERATURE REVIEW

1.1. Aim of thesis

This thesis aims to investigate the effects of the addition of bromelain in a high-fat and high-protein diet in laboratory rats. The study evaluated the effect of high and low lipid intake and the role of bromelain on body weight, biochemical markers of tissue damage, oxidative stress, and serum lipids, urea, and creatinine, and gene expression of key digestive enzymes (lipase, pepsin, and trypsin).

1.2. Foods and nutritional systems

Diets and nutritional systems are fundamental pillars of human health and quality of life. Food is not just a source of calories, but rather a complex network of macro- and micronutrients and enzymes that work together to maintain healthy growth, metabolic balance, and prevent chronic diseases. The World Health Organization reports that unhealthy diets are responsible for approximately 11 million deaths annually worldwide, most of which are due to heart disease, stroke, and diabetes (WHO, 2021b). Protein is at the forefront of macronutrients, which is the building block for tissue construction and repair, and a component of hormones, enzymes, and neurotransmitters. Global recommendations have set the daily intake for adults at approximately 0.8 grams per kilogram of body weight, but recent studies have shown that increasing intake to 1.2–1.6 g/kg/day helps maintain muscle mass in older adults and reduces age-related muscle loss by up to 40% (N.R.C., 2023). Increasing dietary protein is also associated with increased satiety and a reduction in energy expenditure of 440 calories per day (Phillips et al., 2016). In contrast, animal protein typically provides all essential amino acids at high quality, while plant protein requires diversified sources to achieve the same efficiency.

Fat is a concentrated source of energy, providing 9 calories per gram. It plays a role in the formation of cell membranes and the transport of fat-soluble vitamins such as A, D, E, and K. The World Health Organization recommends that healthy fats constitute 20–35% of total daily energy, with saturated fats being less than 10%. Multiple studies have shown that unsaturated fatty acids, especially omega-3 and omega-6, reduce inflammation and reduce the risk of heart disease by 15–20% when consuming 250–500 mg of DHA and EPA daily, while excessive consumption of saturated fats and trans fats increases the risk of heart disease by up to 25% (Mozaffarian et al., 2014). nutritional studies for children have recommended that fats constitute 40% of energy in the early years to support neurodevelopment, along with 100 mg/day of DHA (Savarino et al., 2021).

This system is complemented by enzymes, specialized proteins that catalyze thousands of vital reactions and are indispensable to the body. They digest carbohydrates (amylase), fats (lipase), and proteins (protease), converting them into smaller, absorbable units. Their activity also depends on cofactors such as zinc, selenium, iron, and B vitamins. Previous studies have shown that selenium deficiency has been associated with a 32% increased rate of respiratory infections, while zinc is involved in more than 300 vital enzyme reactions, and its deficiency leads to delayed growth and weakened immunity (Tourkochristou et al., 2021).

Micronutrients and vitamins are the backbone of these interactions. Vitamin D deficiency, which affects more than a billion people worldwide, has been linked to a 20% increase in heart disease. Vitamin E acts as a powerful antioxidant that protects membranes from damage. B vitamins play a pivotal role in amino acid metabolism and lowering homocysteine levels. A clinical trial showed that B12 and folate supplements reduced homocysteine levels by 30% (Hooper et al, 2020). Iron is essential for hemoglobin formation and oxygen transport, and its deficiency is the most common cause of anemia, affecting approximately 1.6 billion people, or 24% of the world's population (WHO, 2022).

Studies indicate that enhancing the diet with comprehensive micronutrients contributes to significantly improves health indicators. A trial of 1,200 adults showed that taking a multivitamin and mineral supplement for 24 weeks reduced harmful cholesterol levels

by 12% and improved insulin sensitivity by 9% (Kaput and Monteiro, 2024). Another study showed that increasing ultra-processed food consumption by 10% of total energy intake was associated with a 14% increased risk of all-cause mortality (Fang, 2024). This demonstrates that diets are not simply a means of satisfying hunger or providing energy, but rather an integrated network that determines the path to health and quality of life. A balance of high-quality proteins, healthy unsaturated fats, essential vitamins and minerals, and active enzymes is key to reducing the risk of chronic diseases and enhancing immunity.

1.3. Free fatty acids

Free fatty acids (FFAs) are important biomolecules that originate either from the breakdown of triglycerides stored in adipose tissue or from food. They play a key role in providing the body with energy and regulating metabolism. FFAs are typically found in the bloodstream bound to albumin, a major fuel source, especially during fasting and exercise. Despite the importance of fatty acids in the diet, chronically elevated levels of FFAs are associated with several metabolic disorders, such as insulin resistance, type 2 diabetes, heart disease, and weight gain. One study showed that individuals with high levels of FFAs were 50% more likely to develop insulin resistance than those with normal levels (Boden, 2008). Other research has shown that increased plasma FFA concentrations inhibit insulin signaling in the liver and muscles, contributing to increased hepatic glucose production and reduced muscle glucose uptake, thus leading to elevated blood sugar (Shulman, 2014). FFAs play a role in regulating gene expression by activating nuclear receptors such as PPARs, which control lipid and glucose metabolism, making them an important focus in obesity and metabolism research. A clinical study showed that lowering FFA levels using drugs that stimulate fat storage in adipose tissue improved insulin sensitivity by 20% over 12 weeks (Tourkochristou et al., 2021). The World Health Organization also reports that elevated free fatty acids are a risk factor contributing to heart disease. Increased FFAs are associated with increased lipid peroxidation within cardiac cells and elevated oxidative stress, leading to cardiomyopathy. Conversely, maintaining balanced levels of FFAs is essential, as they are a primary source of energy during periods of prolonged starvation and are important in supporting brain function and vital organs (WHO, 2021a). All studies have confirmed that controlling FFA levels through healthy lifestyle choices,

such as regular physical activity and a balanced diet, reduces the risk of metabolic syndrome and diabetes.

1.4. Lipid

Lipid is one of the most important of nutrients that provides the body with high energy density (≈9 kcal/g) and contributes to the structure of cell membranes, hormone balance, and the transport of fat-soluble vitamins (A, D, E, and K). Nutritionally, they are divided into saturated fatty acids (SFA) and monounsaturated/polyunsaturated fatty acids (MUFA/PUFA). A balanced consumption favors unsaturated fatty acids to support heart health, as increased consumption of saturated fats has been associated with increased cardiovascular risk factors. Ghee, a concentrated form of milk fat, is one of the most prominent examples of fats. It is rich in SFAs, with moderate amounts of MUFA and CLA. In a randomized trial in adults, replacing the daily fat portion with ghee compared to olive oil increased ApoB by 0.09 g/L (95% CI 0.02–0.17) and increased non-HDL cholesterol by 0.53 mmol/L (95% CI 0.01–1.05) without significant differences in TAG, glucose, or insulin resistance, supporting the recommendation to replace saturated fats with higher unsaturated fats to reduce cardiovascular risk (Hosseinabadi, 2022). In contrast, at lower concentrations and in contexts comparable to hydrogenated oils, a randomized clinical trial of 129 participants for 40 days reported that the ghee group showed a decrease in triglycerides (TG) and an increase in apo-A/HDL-C, while non-hydrogenated liquid oils achieved a broader reduction across the lipid spectrum compared to hydrogenated oils. This suggests that ghee, at moderate doses, may have a less negative effect than industrial trans fats containing trans fatty acids (TFAs), but it does not compare favorably with liquid vegetable oils in improving overall lipid profiles (Chinnadurai, 2013). Meanwhile, those who consumed mustard oil had a 50.9% higher risk of coronary artery disease compared to those who consumed ghee (OR≈0.49 in favor of ghee), with a positive association between increased mustard oil intake and increased TG, TC, LDL, TC/HDL, and LDL/HDL ratios (M. V Kumar, 1999). At higher concentrations or modified formulations, animal data show that ghee enriched with CLA reduced plasma cholesterol by ≈52% and triglycerides by ≈30%, increased HDL by ≈33%, and decreased the atherogenic index from 0.472 to 0.244, with significant improvements in antioxidant enzymes (CAT ↑27%, SOD ↑130%, GST ↑168%) and reduced organ lipid deposition (Manna et al, 2016). Another study in rats

fed a diet containing 10% ghee for 4 weeks had no significant effect on total cholesterol, but increased TG and did not increase hepatic oxidative stress (Mohammadifard et al, 2010). Recent research suggests that its effect on weight, liver, and kidney function may not necessarily be negative when consumed in moderation and within the context of a balanced diet. In a study in 2018, 150 Indian adults, researchers observed that consuming 10–15 grams of ghee daily over a 3-month period did not result in an increase in body mass index (BMI) or waist circumference. Some participants even experienced a slight decrease in visceral fat, 4.7% compared to the control group that consumed vegetable oil (Dhibi et al., 2011). Regarding liver function, an animal study showed that rats receiving Ghee at 10% of their daily calories for 8 weeks showed no elevation in liver enzymes (ALT and AST) compared to the control group. Instead, hepatic triglyceride levels were reduced by 18.3%, suggesting a potential protective effect against non-alcoholic fatty liver disease (NAFLD) (Aldabbagh et al., 2022). Regarding kidney function, another study found that rats which fed a diet containing 10% local ghee for 4 weeks showed significant changes in kidney function parameters. Serum creatinine levels increased from 0.35 ± 0.02 mg/dL in the control group to 0.49 ± 0.04 mg/dL in males and 0.52 ± 0.05 mg/dL in females (Bukhari et al., 2020).

1.5. Amino acids

Amino acids are the building blocks of proteins. They are small organic molecules containing an amino group and a carboxyl group. They play a pivotal role in building tissues, regulating enzymes and hormones, and supporting metabolic processes. The human body contains 20 major amino acids, nine of which are classified as essential amino acids that must be obtained from food, such as leucine, isoleucine, valine, tryptophan, methionine, phenylalanine, threonine, and histidine. World Health Organization reports indicate that a lack of protein or amino acid intake in the diet can lead to slow growth in children and increased rates of infectious diseases, eggs are the cheapest and best example of a protein source that is rich and balanced in terms of amino acid composition. They contain all essential amino acids, making them a global standard for assessing protein quality (WHO, 2021a). Egg whites are characterized by the presence of albumin protein, which contains a group of essential amino acids such as leucine, valine, and threonine, making it highly bioavailable and associated with immune support and muscle growth. Studies have shown that albumin protein

contributes to enhanced nitrogen balance and improved amino acid utilization efficiency compared to lower-quality protein sources (Smith et al, 2018). Research has also indicated that branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine play a particular role in stimulating muscle protein synthesis. A previous study showed that a dose of 2–3 grams of leucine as part of a protein-rich meal stimulated muscle protein synthesis by 33% compared to low-leucine meals (Churchward-Venne et al, 2014). Aromatic amino acids such as tryptophan are a major precursor to the neurotransmitter serotonin, leading to their association with mood and sleep regulation. In contrast, chronic excess levels of certain amino acids, such as BCAAs, in the blood have been associated with insulin resistance and an increased risk of type 2 diabetes. A previous study showed that individuals with high levels of BCAAs in their diet had a 60% higher risk of developing diabetes over a 10-year follow-up period (T. J. Wang et al, 2011). Amino acids not only serve as an alternative fuel during starvation or prolonged exercise, as they can be broken down to produce glucose via gluconeogenesis in the liver. Some amino acids are also used as therapeutic agents. Arginine, for example, enhances nitric oxide production and improves blood flow, while glutamine is essential for immune and intestinal cells. Another study showed that administering glutamine to patients in intensive care units reduced infection rates by 21% (Wischmeyer et al, 2014). Furthermore, amino acids act as regulators of gene expression and cellular signaling through pathways such as mTOR, which controls cell growth and proliferation. Evidence points to the importance of a balanced diet of amino acids, as a deficiency in one amino acid can disrupt the functioning of others, known as a "limiting amino acid." Therefore, it can be said that amino acids represent a fundamental pillar of health. They are not merely components of protein, but rather active elements in metabolic, immune, and neurological balance. Obtaining them from diverse and balanced food sources, such as eggs and especially albumin protein, is a necessary step to maintaining optimal health.

1.6. Proteins

Protein is one of the most important macronutrients that the body cannot do without. It consists of chains of amino acids from which almost all body tissues are built, forming the building blocks of muscle, skin, hair, enzymes, hormones, and immune factors. Protein differs from carbohydrates and fats in that it is the body's primary source of

nitrogen, making it crucial for maintaining nitrogen balance and normal growth. The World Health Organization's recommendations indicate that the basic daily requirement for protein in adults is approximately 0.8 grams per kilogram of body weight, however, a growing number of studies have shown that increasing consumption to 1.2–1.6 grams/kg/day can provide additional benefits, including preserving muscle mass, reducing visceral fat, increasing satiety, and improving weight control. A systematic review showed that high-protein diets reduced LDL cholesterol by 0.2–0.3 mmol/L and slightly increased HDL cholesterol, along with improved insulin sensitivity in some clinical groups (Matsuoka et al, 2022). These findings reinforce the view that protein is not only a building block but also plays a role in metabolic improvement and long-term health. It is important to focus on protein sources with high nutritional value. Among the protein sources with high biological value, albumin, extracted from egg white, stands out. It constitutes the main protein in the egg white, accounting for approximately 54% of its total protein, in addition to other proteins such as ovotransferrin, lysozyme, and ovomucoid. The egg white represents approximately one-third of the egg's weight (≈ 33 grams) and contains an average of 3.6 grams of high-quality protein with a biological value of approximately 100, meaning that all essential amino acids are present in approximately balanced quantities. What distinguishes egg protein is that it is digested at a moderate rate and leads to an effective increase in muscle protein synthesis rates after exercise. Several reviews have considered it an ideal source for preventing sarcopenia in the elderly and for supporting athletes in improving muscle strength and performance (Puglisi et al, 2022). From a practical perspective, nutritional studies have shown that different albumin concentrations in the diet have varying effects. A recent study on wheat bread fortified with egg albumin at concentrations of 5, 15, and 20% of dry matter showed that adding 20% albumin significantly reduced protein digestibility in vitro, from 62.6% in regular bread to 43.1% in fortified bread. This was accompanied by a significant decrease in the slowly digestible starch (SDS) fraction. This suggests that excessive albumin addition may negatively affect the digestibility and absorption of some components (Rosas-Rivas et al, 2025). In contrast, low-dose additives (5–15%) had a positive effect on bread texture and physical properties without significantly reducing digestibility, suggesting that moderate use of albumin in processed foods can improve nutritional value and technological properties without significant adverse effects. Furthermore, in clinical

studies testing egg white as a nutritional supplement in the elderly, results showed that consuming 20–70 g/day of egg white protein-containing formulas for 8–16 weeks resulted in a significant increase in serum albumin levels and improved overall nutritional status (Detarun et al, 2022). This finding is clinically important because low serum albumin is an indicator of malnutrition and high mortality rates in the elderly and chronically ill**.**

Another study confirmed that egg proteins also contribute to enhancing immunity, antioxidant functions, and regulating inflammation via compounds such as ovotransferrin. Thus, it is clear that egg protein, particularly albumin, represents a model of a complete dietary protein; at moderate concentrations, it can contribute to improved muscle performance and metabolic functions, while at very high concentrations, it may exhibit some limitations in digestibility (Batiha et al, 2021).

1.7. Enzymes

Enzymes are specialized protein molecules that act as biocatalysts, accelerating chemical reactions in cells without being consumed by them. They are characterized by their ability to reduce the activation energy required to initiate a reaction, allowing biological reactions to occur quickly and efficiently at temperatures and pressures suitable for life. The importance of enzymes is not limited to digestion alone, but extends to hundreds of processes such as energy metabolism (enzymes of the mitochondrial respiratory chain), DNA replication and repair (DNA polymerases), cellular transport and signaling, and immune defense via enzymes such as lysozyme. Recent research confirms that enzyme imbalance, inhibition, or stimulation is associated with a wide range of diseases such as cancer, liver and kidney diseases, neurological disorders, and cardiovascular disease. In the field of nutrition, studies have focused on digestive enzymes such as lipase, pepsin, and trypsin, which control the digestion of fats and proteins. In an experimental study on rats fed a high-fat diet, lipase gene expression increased 149-fold compared to the control group, while adding bromelain (a pineapple extract) resulted in an additional 427-fold increase, reflecting its role in enhancing fat digestion and improving nutrient utilization (Hooper et al, 2020). Conversely, on high-protein diets, pepsin gene expression increased 15.7-fold compared to the control group. However, upon the addition of bromelain, expression dropped sharply to only 0.18,

suggesting that bromelain may play an alternative role in protein digestion, reducing the need for endogenous pepsin production. The same was observed with trypsin, whose expression increased 65.7-fold with high protein, then decreased to 0.44 upon adding bromelain. These results highlight that exogenous enzymes can regulate and modulate the responses of endogenous enzymes, achieving a better balance between digestion and metabolism. In the clinical and medical context, the role of enzymes as indicators of vital organ functions is highlighted. For example, liver enzymes such as ALT (GPT), AST (GOT), and ALP are used as accurate diagnostic indicators of liver health, as their elevation reflects hepatocellular damage or bile duct obstruction. A clinical trial on 120 patients with non-alcoholic fatty liver disease (NAFLD) showed that diet and nutritional intervention programs led to a significant reduction in ALT and AST levels, demonstrating that enzymes are not merely digestive tools but rather pivotal biomarkers for monitoring the disease (Mohammadifard et al, 2010). In other research on oxidative stress, it was observed that levels of antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) decrease with an unhealthy diet and rise again with interventions with antioxidants or digestive enzymes, further linking enzymes to the immune response and protection of cells from damage. Enzymes have also been widely used in the pharmaceutical and food industries, such as amylase in baking and beverages, and pepsin in protein extraction. A systematic review demonstrated that enzymes extracted from plant or animal sources can play supportive roles in general health, including reducing inflammation, improving digestion, and aiding in the absorption of micronutrients (Batiha et al, 2021). These applications reflect the dual importance of enzymes as natural biological agents and as nutritional or therapeutic supplements in medical practice.

1.8. Bromelain enzyme

Bromelain is a mixture of proteolytic enzymes extracted primarily from the stem and fruit of the pineapple. It is a plant enzyme that has gained widespread importance in medical, food, and industrial applications. Bromelain is characterized by its ability to break down peptide bonds in proteins, giving it digestive properties when taken orally and its anti-inflammatory, anticoagulant, and immunomodulatory properties. It has been used for decades in traditional medicine to treat infections and wounds. Still, recent studies have demonstrated its effectiveness in much broader areas, making it a plant

enzyme with high functional value. In digestion and metabolism, animal experiments have shown that adding bromelain to a high-fat diet increased lipase gene expression by 427 fold compared to the control group. In contrast, the increase with a high-fat diet alone was only about 149-fold. This demonstrates bromelain's ability to enhance fat digestion and improve the efficiency of nutrient absorption. With proteins, it reduced pepsin and trypsin gene expression compared to a high-protein diet alone, suggesting that it plays an alternative role in protein breakdown and reduces the burden on the digestive system. These results highlight the dual effect of bromelain in improving digestive efficiency and regulating endogenous enzyme response. In clinical and human trials, a pilot study demonstrated that rats fed a high-fat, high-protein diet showed significant increases in liver enzymes (ALT, AST, ALP), decreases in antioxidants such as GPx, and increased markers of oxidative stress such as MDA. However, with the addition of bromelain at a dose of 4 mg/day, these enzymes decreased significantly, and liver and kidney tissues returned to near-normal levels, with increased GPx levels and decreased MDA, reflecting its role in reducing oxidative damage and improving vital organ function. Regarding inflammation and the immune system, a systematic review demonstrated that bromelain can inhibit inflammatory pathways (such as NF-κB and COX-2) and reduce the production of pro-inflammatory cytokines such as TNF-α and IL-6. A clinical trial in patients with osteoarthritis showed that taking bromelain supplements at a dose of 500–750 mg daily for six weeks resulted in improvements in pain and mobility indicators equivalent to the effectiveness of nonsteroidal anti-inflammatory drugs (NSAIDs) but with fewer side effects (Brien et al, 2004). Other studies have shown that bromelain enhances innate immunity by increasing the activity of macrophages and natural killer cells (NK cells), making it helpful in fighting inflammation and infection. Anticoagulant properties have also been reported, as bromelain inhibits platelet aggregation and reduces fibrin levels, thus preventing the formation of blood clots. In another clinical trial, bromelain was found to improve blood fluidity and reduce the risk of clots in patients with cardiovascular disorders when taken with conventional anticoagulant medications. This property, in addition to its anti-inflammatory effects, has made it a subject of great interest in cardiovascular research, and it has been nominated for use in the pharmaceutical industry as a treatment for inflammation and edema (Rathnavelu et al, 2016). Research also indicates its potential role as an adjuvant in cancer treatment, as it has been shown to increase the

permeability of cell membranes and enhance the absorption of certain chemotherapeutic drugs. There are preliminary studies on its role in inducing apoptosis in certain cancer cell lines. Accordingly, bromelain is considered a multi-beneficial plant enzyme, combining digestive, anti-inflammatory, anticoagulant, and immune-supporting effects, with potential future applications in cancer treatment and improving drug absorption (Pavan et al, 2012).

1.9. Digestive enzymes

Proteases, such as pepsin, trypsin, and chymotrypsin, are among the most important digestive enzymes responsible for protein breakdown. Their activity is directly influenced by diets high in protein or fat. In aquatic models, it was observed that high dietary protein led to an increase in the mRNA concentrations and activity of trypsin and pepsin, suggesting a transcriptional response linked to the protein content of the diet (Wang et al., 2006). The experiments in poultry showed that supplementing with exogenous proteases at doses ranging from 80 to 160 mg/kg of feed reduced pancreatic trypsin activity by 35–47% and reduced its gene expression by 19–37% after 42 days which suggesting a feedback mechanism that reduces the need for endogenous secretion when exogenous digestive enzymes are available (Yuan et al., 2015). A study also showed that reducing dietary protein to 7% for 8 days resulted in significant reductions in trypsin, chymotrypsin, and elastase activities compared to normal levels (Lhoste et al., 1993). Regarding lipids, a human study showed that a high-fat diet altered the gene expression of approximately 35 pancreatic genes compared to a low-fat diet, including genes associated with digestive enzymes, suggesting a sensitivity of the pancreas to dietary fat content (Ricketts and Brannon, 1994). In the experiments with bromelain supplements, it has been shown that it increases pancreatic trypsin activity in the digestive tract, and other studies in fish have shown that adding it to feed increases pepsin activity by approximately 26–29 IU/g, reflecting the ability of exogenous enzymes to enhance digestive efficiency or influence endogenous enzyme expression (Kostiuchenko et al., 2022). High-protein diets increase the activity and expression of proteinase enzymes. In contrast, high-fat diets lead to changes in gene expression patterns, particularly lipase, which is primarily responsible for the breakdown of dietary fats into fatty acids and glycerol, facilitating absorption and energy utilization (Margolis et al., 2016). Pancreatic lipase works synergistically with bile salts to digest fats and

also plays a crucial role in cholesterol metabolism through lecithin cholesterol acyltransferase (LCAT), which regulates the transport of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Connelly, 1999). Physiologically, normal lipase levels range from 0 to 160 U/L, and elevations above threefold often indicate pancreatic dysfunction, particularly acute pancreatitis, where levels may be 5–10 times higher than the reference range (Pezzilli et al., 1993). In an animal study on mice and similar models, it was found that a high-fat diet increased the gene expression level (mRNA) of the lipase enzyme by about 39% after 3 days of the experiment, followed by a 33% increase after 13 days and a further increase of about 26% after 57 days (Rippe et al., 2003).

1.10. Effect of high-fat diet on body

A high-fat diet (HFD) is defined as a dietary pattern that contains more than 45% of daily calories from fat, and is often associated with high consumption of saturated fat or trans-fat. Several studies have indicated HFD's adverse effects on metabolic health, as it is considered a significant catalyst for weight gain and obesity due to the high number of calories provided by one gram of fat (9 calories per gram of fat) (Thom and Lean, 2017). A study conducted on laboratory rats confirmed an average weight gain of 32.7% in rats fed a HFD diet compared to the control group after 14 weeks, with a 41% increase in visceral fat accumulation compared to subcutaneous fat (Ghose et al., 2011). At the liver enzymes (ALT and AST) were elevated by 58% and 47%, respectively, lipid accumulation in hepatocytes increased by 63% after 8 weeks, and activation of the NF-κB inflammatory pathway in the liver was increased by 3.2-fold compared to a normal diet (D’souza et al., 2012). In the kidney, a 19% decrease in glomerular filtration rate (GFR) was observed after 16 weeks of HFD in animal models, it's had an increase of 210% in proteinuria (albuminuria), and a 2.8- and 3.1-fold increase in the activation of genes associated with renal fibrosis (TGF-β1 and COL1A1) (Kundu et al., 2022). At the level of gene expression of digestive enzymes, a 37% decrease in the expression of the pancreatic lipase (PNLIP) gene was recorded, and the expression of the amylase gene (AMY2A) was inhibited by 29% with a change in the methylation pattern of digestive genes, as methylation on the lipase gene promoter increased by 61% (Baek et al., 2018).

1.11. The effect of high-protein diet on body

A high-protein diet (HPD) is defined as one that provides more than 25–30% of daily calories from protein, or 1.5-2.5 grams/kg of body weight per day. A common pattern in diets aimed at weight loss or muscle building. While many studies demonstrate short-term benefits of this diet in improving body composition and reducing body fat, recent evidence suggests potential side effects on the liver and kidneys, an increased risk of oxidative stress, and altered gene expression of digestive enzymes such as pepsin, trypsin, and lipase, which could disrupt digestive balance in the long term. At the weight level, a randomized study showed that participants who consumed 30% of their calories from protein lost an additional 4.3 kg over 12 weeks compared to the moderate protein group (20%), with better preservation of muscle mass (Leidy et al., 2015). In an experiment on rats, a high-protein diet (50% of calories) led to a 28% reduction in visceral fat accumulation after 8 weeks, but increased liver mass by 12% (Manfredi-Lozano et al., 2016), indicating that weight loss may be accompanied by undesirable structural changes in organs. At the liver level, a study on 37 healthy participants showed that consuming 3 g/kg/day of protein for 4 weeks increased ALT levels by 19% without changing AST, indicating mild liver stress (Mardinoglu et al., 2018). In an animal model, a high-protein diet increased fat accumulation in the liver by 34% when using only animal proteins, while this was not observed with plant proteins (Shibuya et al., 2020), confirming that the protein source has a decisive influence in determining metabolic effects. Regarding kidney function, a systematic review of 10 trials found that HPD increased glomerular filtration rate (GFR) by 10–15% in healthy individuals, but increased urinary albumin excretion by 35% in patients with type 2 diabetes (Friedman et al., 2012). Another study showed that protein consumption >1.8 g/kg/day was associated with a 1.2 ml/min/1.73 m² lower annual GFR compared to moderate consumption (0.8–1.2 g/kg/day) suggesting a risk of progressive functional decline with chronic use (Knight et al., 2003). Regarding oxidative stress, a study in mice showed that a high-protein diet (45% protein) increased liver peroxide levels by 42% and decreased reduced glutathione (GPx) by 27% after 12 weeks (Camiletti-Moirón et al., 2015). In another clinical study in 25 athletes, consuming 2.5 g/kg/day of protein for 8 weeks increased plasma markers of oxidative stress (8-OHdG and MDA) by 22–31% (Poortmans and Dellalieux, 2000), indicating that even healthy individuals are not

immune to this risk. Finally, at the level of gene expression of digestive enzymes, a molecular study on human pancreatic cells (in vitro) showed that exposure to high concentrations of amino acids (mimicking HPD) reduced gene expression of PNLIP (lipase) by 40% and PRSS1 (trypsin) by 35%, while increasing PGC (pepsin) by 18% (Yanagisawa, 2023). In laboratory rats, a high-protein diet reduced intestinal lipase activity by 33% after 6 weeks, with transcriptional inhibition of the PNLIP gene promoter via the mTORC1 pathway (Kok et al., 2022). This indicates that excessive protein intake not only burdens the liver and kidneys but also disrupts the molecular mechanisms responsible for digestion, which may lead to chronic digestive disorders. Overall, despite the short-term benefits of a high-protein diet for weight control and muscle mass maintenance, recent evidence suggests potential risks to the liver (increased ALT and lipid accumulation) and the kidney (elevated urinary albumin and long-term GFR decline), as well as the induction of oxidative stress in tissues. Importantly, this diet disrupts the gene expression of digestive enzymes, particularly lipase and trypsin, which may impair digestion in the long term. These findings highlight the need to tailor protein intake to health status and avoid chronic excess, especially in individuals predisposed to liver or kidney disease.

1.12. Effect of bromelain enzymes on body

Bromelain, an enzyme extracted from pineapple, combines proteases (proteolytic enzymes) with anti-inflammatory, anticoagulant, and digestive-stimulating properties. It has been used for decades in complementary medicine to treat inflammation, post-surgical swelling, and digestive disorders (Kansakar et al., 2024). Studies indicate that bromelain's role is not limited to digestive aids, but extends to influence weight regulation, protecting the liver and kidneys from oxidative stress, and modifying the gene expression of digestive enzymes. Regarding weight, a study on obese rats showed that consuming 10 mg/kg/day of bromelain for 8 weeks reduced average weight by 18.3% compared to the control group, while reducing visceral fat accumulation by 27% (Varilla et al., 2021). Gene expression of the lipid peroxidation receptors PPAR-α and CPT1A in the liver was also increased by 2.1- and 1.8-fold, enhancing fat burning (Ramos-Lopez, 2025). At the liver level, another study showed that bromelain reduced ALT and AST levels by 34% and 29%, respectively, in models of non-alcoholic fatty liver disease, while reducing hepatic fat accumulation by 41% through inhibiting the

SREBP-1c pathway that stimulates lipid synthesis (Lee et al., 2025). It also increased glutathione peroxidase (GPx) activity by 52%, indicating enhanced antioxidant defense in liver cells (El-Demerdash et al., 2022). In the kidneys, bromelain has been shown to be effective in a model of diabetic nephropathy, reducing plasma creatinine levels by 22% and urinary albumin excretion by 38% after 6 weeks of treatment, while inhibiting TGF-β1 gene expression in kidney tissue by 45%, indicating a protective effect against renal damage (Hidayat et al., 2023). Regarding the gene expression of digestive enzymes, Huang et al (1996) found that exposure to 50 µg/ml bromelain increased trypsin secretion by 26.7% ± 3.2% compared to the control group. Similarly, an animal study in rats showed that oral administration of bromelain (10 mg/kg/day for 7 days) did not alter gastric pepsin activity levels, but reduced markers of mucosal inflammation by 41%, which may suggest an indirect protective effect on the gastric mucosa (Hale et al., 2005).

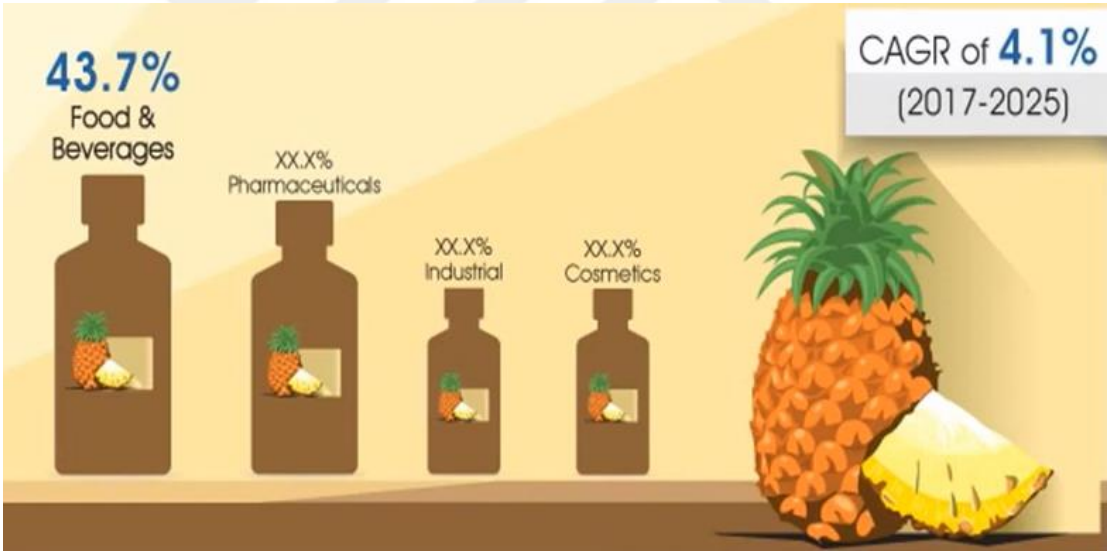


Figure 1.1. Applications of Bromelain enzymes extracted from pineapple (Setthayanond et al., 2025)

1.13. Effects of diets on the oxidative stress

High-fat and high-protein diets disrupt oxidative balance by promoting excessive free radical production, which induces oxidative stress and damages cellular membranes, proteins, DNA, and gene expression. Diets directly affect oxidative stress in cells via metabolic pathways that deplete the body's glutathione stores and increase levels of damage to living tissues. A study conducted on rats showed that a high-fat diet (HFD)

led to a significant increase in oxidative stress indicators, including elevated levels of Thiobarbituric Acid Reactive Substances (TBARS) and 4-Hydroxynonenal (4-HNE), while reduced levels of Reduced Glutathione peroxidase (GPx) in the liver decreased, indicating a severe disturbance in oxidative balance (García-Ruiz et al., 2014) On the other hand, a high-protein diet (HPD) showed a different effect in the brain; Although glutathione peroxidase (GPx) and catalase (CAT) activity was elevated in the hypothalamus, GPx and MDA levels were significantly elevated in the cerebral cortex and cerebellum, indicating oxidative damage despite defensive efforts (Żebrowska et al., 2019). In humans, a study observed that body fat accumulation was associated with elevated serum TBARS and other oxidation markers such as 8-epi-$PGF_2\alpha$, with an inverse correlation with adiponectin levels, demonstrating that fat accumulation is a major driver of systemic oxidative stress (Furukawa et al., 2004). These data support that high-fat diets promote oxidative stress, while high protein may provide some significant defenses but does not prevent oxidative damage, emphasizing the need for nutritional balance to avoid oxidative imbalances.

1.14. Effects of diets on gene expression

Experimental evidence suggests that a change in diet composition induces epigenetic reprogramming in the secretory cells of the gastrointestinal tract in response to nutrient availability. A study in rats fed a 60% fat and 20% protein diet for 8 weeks showed a 50% decrease in gene expression of the pancreatic amylase (Amy2a) enzyme compared to the control group fed a standard diet, while gene expression of the pancreatic lipase (Pnlip) enzyme increased by 65% reflecting a functional adaptation to the increased demand for fat digestion (Ma et al., 2017). While a human study showed that participants who followed a low carbohydrate, high-fat, high-protein diet (70% fat, 25% protein) for 4 weeks had approximately 70% reduction in lactase (LCT) gene expression in small intestine biopsies, which is consistent with the significant reduction in lactose and sugar consumption, which may explain the onset of secondary lactose intolerance symptoms in some individuals after returning to dairy consumption (Noakes and Windt, 2017). On the other hand, another study revealed that a high-protein diet (50% of calories) stimulates the gene expression of protease enzymes such as trypsin (PRSS1) and chymotrypsin (CTRB1) in the pancreas by 30–40%. However, it may inhibit the expression of carbohydrate enzymes such as amylase and maltase, suggesting that the

pancreas reallocates its enzyme resources based on nutritional intake (Yan et al., 2025). Another study indicated that a Western diet rich in saturated fat and animal protein stimulates the gene expression of TLR4 receptors in intestinal cells, leading to the activation of NF-κB pathways and increased secretion of inflammatory cytokines, which may indirectly inhibit the gene expression of some digestive enzymes by affecting the intestinal cell environment (Rocha et al., 2016). It is worth noting that the quality of fat and protein plays a crucial role; It was observed that a diet high in unsaturated fats (olive oil, nuts) and vegetable protein (legumes, soy) resulted in less severe changes in the gene expression of digestive enzymes compared to a diet rich in saturated fats and red meat, with amylase and lactase expression levels remaining close to normal (±10%), suggesting that nutrient quality may mitigate negative genetic effects (Tovar et al., 2002). Overall, the HFHP diet induces adaptive changes in the gene expression of digestive enzymes, in line with nutrient availability. However, these changes may become unfavorable upon sudden switching to different diets, or upon long-term continuation, which may lead to pancreatic or intestinal dysfunction.

CHAPTER 2

MATERIAL AND METHOD

2.1. Preparation of laboratory animals

Laboratory rats (white, male Albino rats) were obtained from Tikrit University / College of Veterinary Medicine, a total of 25 rats, with ages ranging between (3-4) weeks and weights ranging between (200-225 g). They were examined by the veterinarian for health status and followed up for a week in terms of health indicators and motor activity. The animals were placed in controlled conditions in terms of ventilation and temperature, ranging between 22-30°C, humidity up to 50%, and lighting period (12:12 hours) inside the animal house. Rats were housed in plastic cages with metal mesh covers and dimensions (12, 15, 29 cm) designed for this purpose. These cages are cleaned and sterilized with 70% ethyl alcohol, and the floor is covered with wooden shavings that are changed twice a week and regularly. The animals are provided with filtered water through the container attached to the cage and designated for this purpose, and the feed allocated to each group is given continuously throughout the actual biological experiment period that lasted for thirty days starting from July to October 2023. Identification cards were placed on each cage in terms of the groups. The animals were placed inside the cages for 10 days before the actual experiment date in order to acclimatize and get used to the place.



Figure 2.1. Preparation of laboratory animals

2.2. Preparing the fodder

While preparing the feed mixtures, care was taken to ensure that they were close to the NRC (1977) values. The feed and nutrient composition were given in Table 2.1

Table 2.1. Contents of the feed used in the experiment

Component	Percentage (%)
Milk casein	20.0%
DL-methionine	0.3%
Cornstarch	15.0%
Granulated sugar	50.0%
Cellulose powder	5.0%
Corn oil	5.0%
Vitamin mix (AIN-76)	1.0%
Mineral mix (AIN-76)	3.5%
Choline bitartrate	0.2%
Total	100.0%
AIN-76 Vitamin Mix	
Component	Percentage (%)
Vitamin A (1,000,000 IU/g)	0.04%
Vitamin D_3 (500,000 IU/g)	0.02%
Vitamin E (50%)	1.00%
Vitamin B_1	0.06%
Vitamin B_2	0.06%
Vitamin B_6	0.07%
Vitamin B_{12} (0.1%)	0.10%
Nicotinic acid	0.10%
Calcium pantothenate	0.16%
Folic acid	0.002%
Biotin (2%)	0.10%
Vitamin K_3	0.0005%
Granulated sugar	98.0695%
Total	100.0%
AIN-76 Mineral Mix	
Component	Percentage (%)
$CaHPO_4 \cdot 2H_2O$	50.00%
NaCl	7.40%
$K_3C_6H_5O_7 \cdot H_2O$	22.00%
K_2SO_4	5.20%
MgO	2.40%
$MnCO_3$	0.35%
$FeC_6H_5O_7 \cdot 5H_2O$	0.60%
$5ZnO \cdot 2CO_2 \cdot 4H_2O$	0.60%
$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$	0.30%
KI	0.01%
$Na_2SeO_3 \cdot 5H_2O$	0.001%
$CrK(SO_4)_2 \cdot 12H_2O$	0.003%
Granulated sugar	11.806%
Total	100.0%

2.3. Experimental design

The biological experiment was conducted in Saladin Governorate -Tikrit, and laboratory experiments were conducted in the central laboratory in Anbar Governorate - Iraq. The trial period extended from July to October 2023. We used 25 albino laboratory male rats divided into five experimental groups (each group containing 5 replicates), with similar body weights. The commercial diet for the rats had similar nutrient content according to NRC (1977). As for fat, the free fat was extracted from cow's milk in the form of free fat (ghee) (Kokotou et al., 2020), and albumin was used as a protein substance (Belinskaia et al., 2020), in addition to the bromelain enzyme extracted from pineapple (Varilla et al., 2021).

T1: Control standard diet only.

T2: Standard diet + high-fat food (ghee) 4 g divided into 2 doses, morning and evening (2 m each).

T3: Standard diet + high protein food (albumin) 4 g divided into 2 doses morning and evening (2 g each).

T4: Standard diet + high-fat foods + bromelain enzyme 4 mg divided into two doses morning and evening (2 mg each).

T5: Standard diet + high-protein foods + bromelain enzyme 4 mg divided into two doses morning and evening (2 mg each).

2.4. Preparatıon of bromelaın enzyme

Bromelain enzyme is isolated and purified from pineapple fruits according to the following steps (Costa et al., 2014)

1- Cutting and grinding**:**

The pineapple is cut into small pieces and ground using an electric blender to obtain a homogeneous paste. A buffer solution containing potassium phosphate is added to protect the enzyme from damage**.**

2- Centrifugation**:**

The paste is placed in a centrifuge at 10,000 rpm for 30 minutes to obtain the raw (upper) liquid containing the bromelain enzyme**.**

3- Sedimentation and separation**:**

Ammonium sulfate is added in a process called salt precipitation, where ammonium sulfate is gradually added to the liquid juice under continuous stirring**.**

4- Concentration and saturation**:**

Ammonium sulfate is added continuously until a concentration of 40-60% is reached, at which point the solubility of the proteins decreases and they precipitate in the solution. The solution is then centrifuged again to separate the precipitate containing the bromelain enzyme. Temperatures must be maintained at 4°C to ensure the enzyme is not damaged**.**

5- Dialysis

A dialysis membrane is used to obtain a highly pure enzyme. This enzyme is used experimentally according to the doses used in the study, while ensuring that it is kept refrigerated to reduce enzyme activity.



Figures 2.2. Preparation of Bromelaın enzyme

2.5. Preparation of ghee

Ghee is extracted from cow's milk according to the following steps (Ray, 2019)

1- Separation stage

Fresh milk is heated until it reaches the boiling point, then left to cool completely to obtain a top layer of fat (cream). This top layer is collected and stored in the refrigerator. This process must be repeated several times to obtain the maximum possible amount of milk fat.

2- Homogenization and whipping stage

Place the cream in a mixer bowl and whisk vigorously and continuously until small pieces of butter are obtained.

3- Collect stage

The solid butter pieces are collected and washed thoroughly with water to remove any remaining curdled milk and impurities, protecting them from potential spoilage.

4- Purification stage

The butter is heated slowly over very low heat to melt the butter and transform it into a golden liquid. A white foam forms on the surface, consisting of water and solids (lactose and casein). These are gradually removed until a clear liquid (ghee) is obtained.

5- Storage

The ghee is left in a tightly sealed glass container and stored at room temperature or in the refrigerator and use as needed.



Figures 2.3. Preparation of Ghee.

2.6. Preparation of albumin

The process of extracting albumin from eggs is similar to the process of extracting other proteins, as it requires controlling the temperature and acidity to preserve it from spoilage, according to (Pereira et al., 2016) as following:

1- Egg white separation stage

Where the egg white is extracted and separated from the yolk, which contains fats and other proteins that may interfere with the purification process**.**

2- Homogenization stage

A slow mixer is used in a glass container with a pH 5.5 potassium phosphate buffer solution added to preserve the protein and prevent protein loss**.**

3- Salt precipitation stage

This step is performed using slow, continuous stirring of 60% ammonium sulfate into the homogenized egg white solution to precipitate the albumin**.**

4- Centrifugation stage

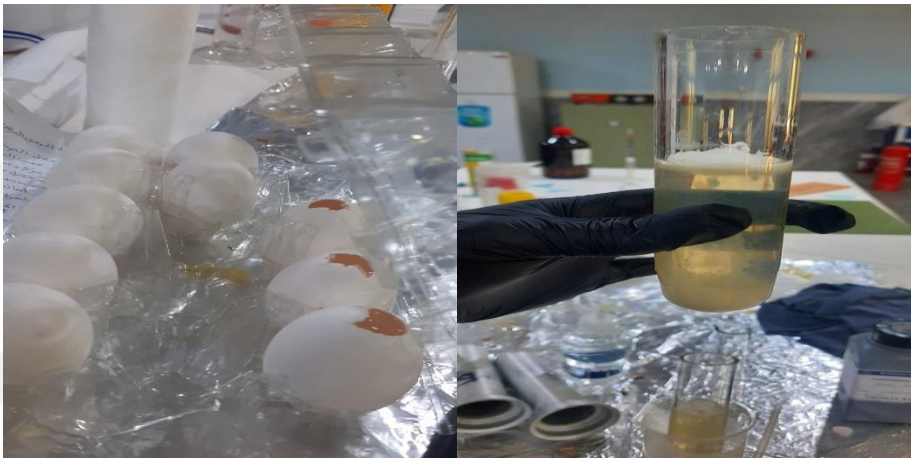
The solution is placed in a centrifuge at 10,000 rpm for 20 minutes**.**

5- Ion exchange purification stage

The precipitate is immersed in a buffer solution and passed through a column containing a charged resin. The albumin adheres to the resin while unwanted impurities pass through. The concentration of the ammonium sulfate solution is then increased to gradually wash the albumin and collect it in separate fractions.

6- Storage phase

Albumin is stored in tightly sealed, clean containers at a low temperature to maintain its effectiveness.



Figures 2.4. Preparation of Albumin

2.7. Feeding of animals

The animals were divided into five groups and were fed manually using a dosing tool. In the protein group (albumin extracted from eggs), 2 ml was given in the morning and 2 ml in the evening. A group of fats extracted from cow's milk. This group was given 2 ml in the morning and 2 ml in the evening. As for the bromelain enzyme group extracted from pineapple, it was given using a microdosing tool : 0.2 ml in the morning and 0.2 ml in the evening (Li et al., 2021).



Figure 2.5. Feeding of Animals.

2.8. Determination of body weight of rats

Rats were weighed weekly and differences between two weeks were calculated. The weight gain of one repetition per week was calculated according to the equation mentioned by (Naji, 2006), which is as follows:

Body weight gain = Average body weight at the end of the week - Average body weight at the beginning of the week

2.9. Preparing and collecting the blood samples

The tools that will be used to draw blood samples from the animals have been prepared after anesthetizing them with chloroform solution (Aledani et al., 2020). After the end of the experiment period, which lasted for 30 days, the animals were starved for 10 hours to ensure the accuracy of the results, then, the animals were anesthetized with ketamine, and after the animals became numb, the animal's limbs were fixed with stabilizers, then, the abdomen was incised longitudinally and blood was drawn through a cardiac puncture. In a size of (3-4 ml) using disposable medical syringes, then part of the blood was transferred to tubes containing EDTA to prevent clotting to conduct blood tests including (PLT - WBC - RBC - HGB). The other part of the blood was transferred to Gel tubes for separating the serum and performing the remaining tests manually.

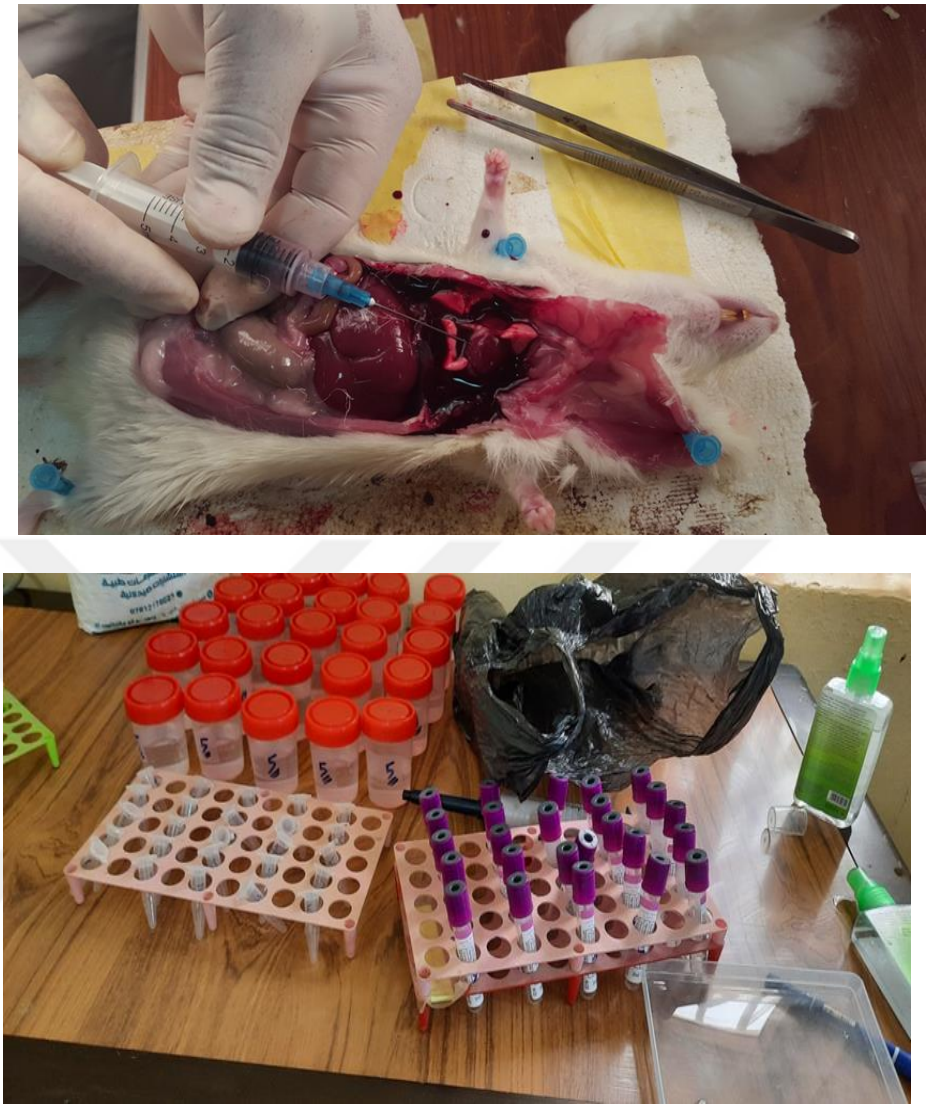


Figure 2.6. Collect blood sample



Figure 2.7. Prepare sampling

2.10. Rat's dissection

Rats were dissected using a dissection kit (Several anatomy) and isolating the organs (liver, kidney) to conduct tissue sections and special examinations, and to determine the extent of the effect of the parameters used in the experiment on them. They were washed with physiological solution (Normal saline) to get rid of blood residues and plankton, then they were placed in a solution of formaldehyde at a concentration of 10% for 24 hours, then it was transferred to a solution of ethyl alcohol at a concentration of 70% for 3 days (University of Alabama at Birmingham, 2023).



Figure 2.8. Animal organs

2.11. Complete blood count examination (CBC)

A blood sample is collected in a tube containing an anticoagulant - usually tubes containing EDTA (ethylenediaminetetraacetic acid) are used - to prevent normal blood clotting. Red and white blood cells and platelets can be counted using a hemocytometer, which is a microscopic slide with a chamber containing a specific volume of diluted blood. The hemocytometer chamber is engraved with a calibration grid to aid in cell counting. The cells in the grid are counted and divided by the volume of blood tested, which is determined by the number of squares calculated on the grid, to obtain the concentration of cells in the sample. Hematocrit can be performed manually by filling a capillary tube with blood, centrifuging it, and measuring the percentage of blood that is made up of red blood cells. Hematocrit can be performed manually by filling a capillary tube with blood, centrifuging it, measuring the percentage of blood that is made up of

red blood cells, then dividing the result by n and subtracting the value of one from it to obtain the blood hemoglobin concentration by using the following equal:

PCV/ 3-1= HGB (Seo and Lee, 2022)



Figure 2.9. Complete blood count examination (CBC)

2.11.1. Determination of white blood cells (WBC)

The same method was used to calculate the number of red blood cells, except for the dilution, which was 20 times, by adding one part of the blood to 20 parts of the Natt and Herick solution. The numbers of blue-coloured white blood cells stained with the colour of the methyl violet dye present in the solution were counted in the four squares. "White blood cells were counted in all four squares of the Neubauer counting chamber, as they appeared dark blue and granular. The total white blood cell count was calculated using the following equation (Natt and Herrick, 1952)**.**

WBC count (cells/µL)of blood = (N/4) x 20 x 10

whereas the N = total number of white blood cells calculated from the four squares.

4 = The number of large squares in which the count was made.

20 = number of dilutions.

10 = To convert the calculated volume of white blood cells from 0.1 mm^3 to 1 ml^3.

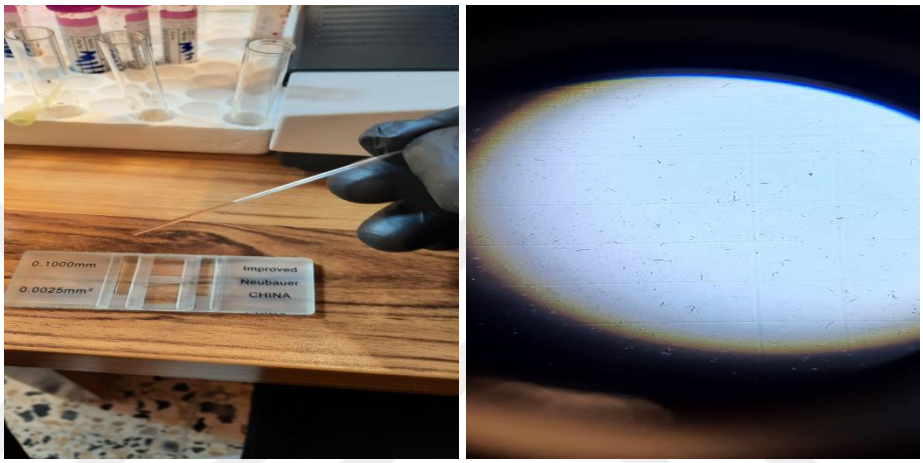


Figure 2.10. White Blood Cells (WBC)

2.11.2. Determination of red blood cells (RBC)

A cell counting process for red blood cells was conducted using a specialized pipette containing a chamber with a red ball. Blood was drawn into the chamber up to the 0.5 mark and then diluted solution until reaching the 101 mark. This indicates a 200-fold dilution of the blood. Gentle pipette agitation was then performed for two minutes to mix the blood with the solution inside the chamber. The first three drops were discarded as they represented only the solution. One drop of the mixture was then placed in the well under the cover glass of the Hemocytometer. The spread of the solution under the cover glass was observed, and after the cells settled, the cell count was determined using a light microscope with a magnification power of X100. Their blue-stained cytoplasm and pink-stained nuclei identified red blood cells. Cell counting was performed within 5 out of 25 squares on the counting chamber. The centre square and the remaining four squares at each corner represented the sample. The following equation was then applied (Zhu et al., 2020)

The number of red blood cells in (1) mm³ of blood= $\frac{N}{5}$ ×25 × 200 × 10

whereas the:

N = total number of red blood cells in five squares.

5 = the number of squares in which the cells were counted.

25=total number of squares.

After completing the tests manually, the results were confirmed using the automated analysis device shown in the pictures below

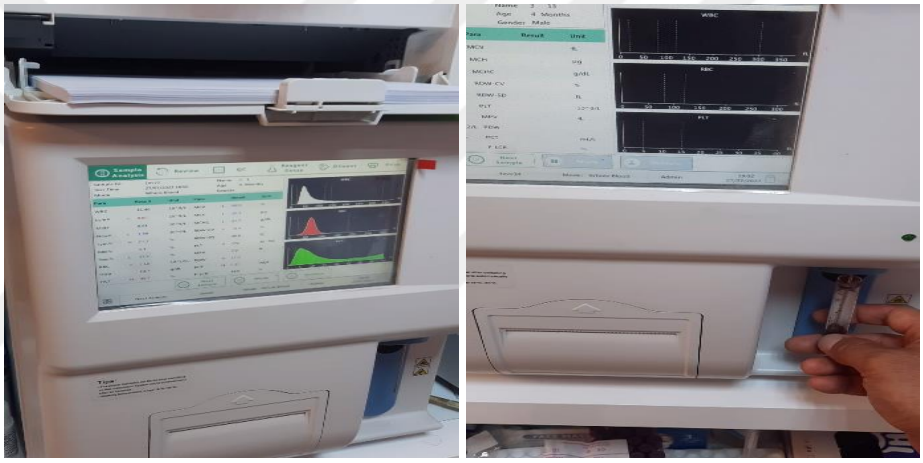


Figure 2.11. Automated CBC device

2.12. Determination of lipids in blood serum

2.12.1. Determination of triglyceride concentration

An examination kit prepared by the Italian company GIESSE was also used, which relies on the enzymatic method to analyze triglycerides into glycerol, as stated in (Lott and Hayton, 1978) after which the following equation was applied for the calculation:

Concentration of triglycerides (mg/100 ml) = absorbance of the sample / absorbance of the standard solution x concentration of the standard solution (200 mg/100 ml).

The results were confirmed using an automatic measuring device, shown in the pictures below



Figure 2.12. Determination of triglyceride concentration

2.12.2. Determination of total serum cholesterol concentration

An examination kit prepared by the Italian company GIESSE was also used, which relies on the enzymatic method to convert cholesterol and cholesterol esters into the dye quinoneimine, as stated (Myers et al., 1976)), after which the following equation was applied for calculation:

Cholesterol concentration (mg/100 ml) = absorbance of the sample/absorbance of the standard solution x concentration of the standard solution (200 mg/100 ml).

The results were confirmed using an automatic measuring device, shown in the pictures below



Figure 2.13. Automatic measuring device

2.12.3.Determination of serum high density lipoprotein concentration (HDL)

The examination kit supplied by the Italian company GIESSE was used based on what was mentioned by (Myers et al., 1976)after which the following equation was applied for the calculation:

HDL concentration (mg/100 ml) = sample absorbance / standard solution absorbance x dilution factor (1.1) x standard HDL concentration (50 mg/100 ml).

2.12.4. Determination of serum low density lipoprotein concentration (LDL)

The value of low-density lipoproteins was extracted using the following equation (Myers et al., 1976)

LDL level (mg/100 ml) = cholesterol concentration - (HDL concentration + triglyceride concentration / 5)

2.12.5. Determination of serum very low density lipoprotein concentration (VLDL)

The value of very low-density lipoproteins was extracted using the following equation (Myers et al., 1976)

VLDL concentration (mg / 100 ml) = triglyceride concentration T.G / 5

2.13. Estimation of kidney function

2.13.1. Determination of serum urea concentration

The concentration of urea in blood serum was estimated based on the method (Wybenga et al., 1971) using the test kit prepared by the Italian company AFLO, and according to the instructions mentioned afterwards, the following equation was applied for the calculation.

Concentration of urea in serum (mg/100 ml) = absorbance of the sample / absorbance of the standard solution x concentration of the standard solution 50 mg/100 ml)

2.13.2. Determination of serum creatinine concentration

Creatinine in blood serum was also estimated using a test kit prepared by the Italian company GIESSE and according to the instructions mentioned by (Lott and Hayton, 1978) after which the following equation was applied for calculation:

Creatinine concentration (mg/dl) = absorbance of the sample / absorbance of the standard solution x concentration of the standard solution (2 g/100 ml).

2.14. Determination of liver enzymes activity

2.14.1. Determination of glutamic oxaloacetic transaminase (GOT)

The GOT enzyme was estimated using the method (Sanghavin and Jivani, 1979), which used pre-packaged analysis kits manufactured by BioSystems -Spain. This method relies on the enzyme's ability to convert aspartic acid into oxaloacetic acid, which spontaneously converts to pyruvic acid. The latter then reacts with the compound 2,4-dinitrophenyl hydrazine (DNPH) to form a reddish-brown collared complex. The absorbance was measured at a wavelength of 546 nanometres and expressed in international units per litter (IU/L).

2.14.2. Determination of glutamic pyruvic transaminase (GPT)

The method involved the use of pre-packaged analysis kits manufactured by Bio systems-Spain. This approach relies on colorimetric measurement to assess the enzyme's activity by measuring pyruvic acid levels derived from alanine; the pyruvic acid reacts with the compound DNPH, forming a reddish-brown colored complex. This complex is measured at a wavelength of 546 nanometres and quantified in international units per litter (IU/L), following the established method (Sanghavin and Jivani, 1979).

2.14.3. Determination of alkaline phosphatase (ALP)

A serum blood sample is collected and mixed with a reagent containing a substrate such as p-Nitrophenyl Phosphate (pNPP) in basic medium. The mixture is incubated at 37°C for a specified period of time, and then the change in absorbance at a wavelength of 405 nm is measured using a spectrophotometer. The result is calculated based on the change in absorbance using an equation or standard curve that is based on a linear relationship between the absorbance and concentration of the standard (Clawin-Rädecker et al., 2021).

2.15. Determination of antioxidant enzymes

2.15.1. Determination of (GPX) in serum

A solution of (0.0017 molar) of hydrogen peroxide H_2O_2 was used, and a solution of (0.0025 molar) of tetra-amino antipyrene and (0.17 molar) of phenol were also used.

The below mixture was then incubated at a temperature of 25°C for 3 to 4 minutes, and 0.1 ml of blood serum was added, and the absorbance was recorded immediately after

addition at a wavelength of 510 nm. The absorbance was repeated after 5 minutes at the same wavelength, according to (Pierce and Tappel, 1978).

A mixture of the following materials was prepared:

1.4 ml		phenol + 4-Amino antipyrine
1.5 ml	0.0017	molar Hydrogen peroxide

2.15.2. Determination of (MDA) in serum

A solution of Thiobarbituric acid (TBA) was used at a concentration of 0.04 molar, and a solution of Trichloro Acetic Acid (TCA) was also used at two concentrations, the first 17.5% and the second 70%. It can be kept in the refrigerator to complete the rest of the samples to be examined. The method of work mentioned by researchers (Guidet and Shah, 1989) was used, where solutions were prepared according to the following table:

Table 2.2. A solution of (TBA) and (TCA)

counter	Samples	Solutions
---	0.15ml	Serum
0.15ml	---	DW
1ml	1ml	(TCA)17.5 % First Con.
The tubes are well blended		
1ml	1ml	% 0.6 (TBA)
The tubes were mixed well and placed in a boiling water bath for 15 minutes, then left to cool.		
1ml	1ml	(TCA) %70 Second Con.
The tubes were left at a temperature of (37°C) for 20 minutes, then the liquid was separated using a centrifuge at a speed of 2000 rpm for 5 minutes, and the absorbance was read at a wavelength of 532 nm.		

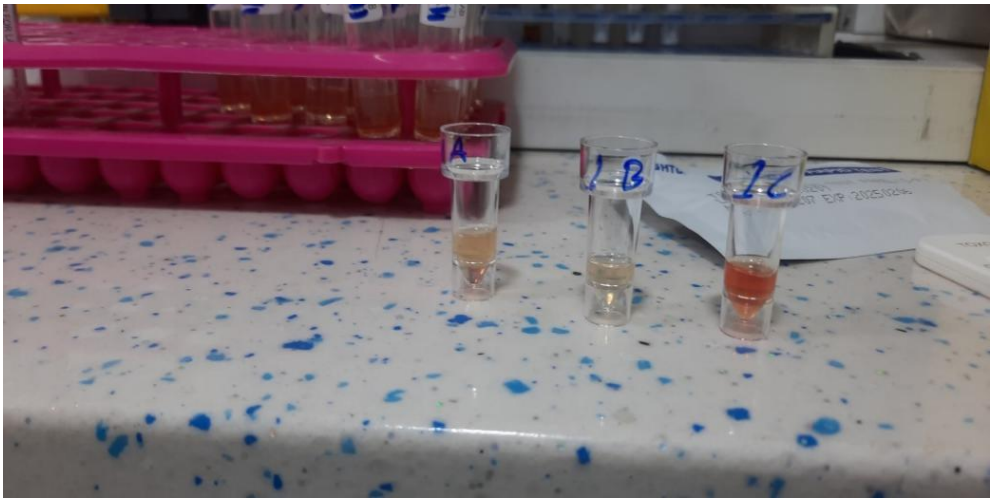


Figure 2.14. Tubes for estimation of (MDA) in serum

Then the following equation was applied for calculation:

MDA concentration mmol/L= Absorbance at 532 nm / E 0 × L x dilution factor

L = light path = 1cm

E0 = 1.56×105 / molar. Poison

mmol= Micromol

2.16. Isolation of polymorphonuclear macrophages/neutrophils (PMNs) from peripheral blood

Polymorphonuclear macrophages were isolated by placing the drawn blood in sterile, sealed plastic tubes containing 500 IU heparin to prevent clotting and dextran solution to precipitate red blood cells. The contents of the tube were then gently mixed. The tubes were then placed in an incubator at 37°C for 45 minutes. The plasma layer containing the white blood cells was withdrawn and transferred to another sterile plastic tube. The white blood cells were washed with Hanks' balanced salt solution twice by centrifugation at 3000 rpm to remove the plasma and heparin. The cells were then suspended in the same solution to reach a concentration of 106 x1 cells/ml. Cell numbers were calculated according to (El-Feki et al., 2001) and cell viability was calculated directly by placing 40 microliters of cells and mixing them with 40 microliters of trypan blue dye and counting 200 cells and extracting viability as in the following equation:

Percentage of cell viability= number of living cells/ Total number of cells x 100

2.17. PMNs cell migration under agarose

The method Adcharamoorthy (1990) was adopted as follows: 10 ml of migration medium was placed in sterile plastic dishes and then left to solidify. Agarose layer was punctured using an 8 mm cork piercer, with two holes for each dish. The hole circle was then marked from the outside. After that, 200 microliters of PMNs cell suspension were placed at a concentration of 1 x10 ^{6} cells/ml in each hole. The dishes were incubated at 37°C in the presence of 5-10% CO_2 for 32 hours. After the incubation period, the dishes were taken out and fixed with methanol for 15 minutes. After the methanol dried, the agarose dishes were stained with Giemsa stain for 20 minutes according to the modified (Papageorgiou, 2013) method. The dishes were washed with distilled water and examined after drying. The diameter of the PMNs cell migration circle was measured using an eye ruler.

Phagocytosis Index (P.I)= Number of phagocytic cells / total number of phagocytic cells (phagocytic + non-phagocytic) X 100

Migration Inhibiting Factor (M.I.F)= Diameter of migration circle in test treatment (mm)/Diameter of migration circle in control treatment (mm) x 100

2.18. Phagocytosis of heat-killed candida yeast.

2.18.1. Preparation of yeast suspension

The yeast Candida albicans was obtained from the Graduate Studies Laboratory/College of Science/University of Anbar and after confirming it by microscopic examination, it was cultured on Sabaroaud broth medium and incubated for three days at 37°C. Then the culture medium was centrifuged at 3000 rpm for 15 minutes. The precipitate containing the cells was washed twice with Hanks' balanced salt solution. The cells were suspended in RPMI-1640 culture medium at a concentration of 106×4 cells/ml. Then the yeast cells were killed by incubating them at 100°C for an hour, then stored at 4°C until use (Saresella et al., 1997).

2.19. Determination of gene expression

2.19.1. RNA extraction by TRIzol™

The extraction of RNA was carried out according to the method (Rio et al., 2010) According to the following steps:

1- Blood suspend it with TRIzol 0.6 ml

2. Fifteen minutes incubation to permit complete dissociation of the nucleoprotein complex .

3. The chloroform was added as 200µl of TRIzol™ Reagent used for lysis .

4. Incubation for 2–3 minutes at room temperature.

5. The sample was centrifuged for 10 minutes at 10,000 rpm. The mixture was separated into a lower red phenol-chloroform, interphase, and a colorless upper aqueous phase.

6. The aqueous phase containing the RNA was transferred to a new tube .

7. The RNA was precipitated by adding 200 µl of isopropanol or absolute ethanol to the aqueous phase.

8. The mixture was incubated for 2minutes at room temperature

9. The mixture was centrifuged for 10 minutes at 10,000 rpm. Total RNA precipitate on the filter of the spin column tube.

10. The supernatant was discarded.

11. The column gets re-suspended by 0.5 ml of washing buffer 1.

12. The tube was centrifuged for 2 minutes at 10000rpm.

13. The supernatant was discarded.

14. The column gets re-suspended by 0.5 ml of washing buffer 2.

15. The tube was centrifuged for 2 minutes at 10000rpm.

16. The supernatant was discarded.

17. The column gets pre-heated elution by 75 µl and centrifuged for 1 min at 10000rpm .

18 .Total RNA samples stored in deep freezer.

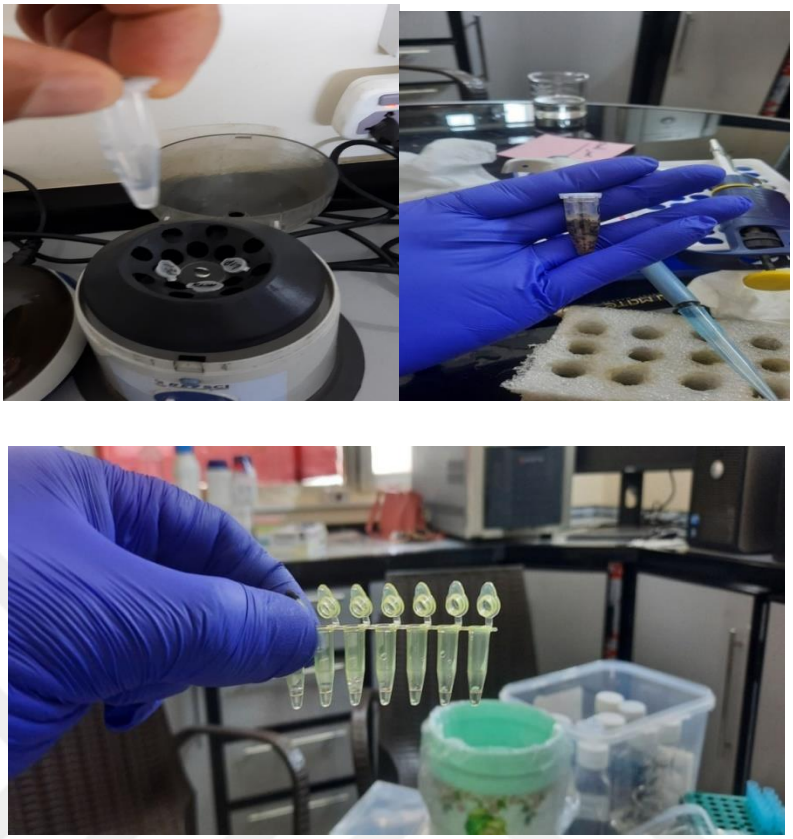


Figure 2.15. RNA extraction by TRIzol

2.19.2. cDNA synthesis

The cDNA was created by cDNA ready to use kit (Bioneer, Korea) by

1. Eighteen microliter of RNA extract was added to microfuge tube

2. Two microliters of hexamer primer (for prokaryotic cells) or oligo dt (for eukaryotic cells) was added and then mixed well

3. the mixture was incubated in PCR with the following circumstance , 37 ºC for 10 min followed 42 ºC for 1hr and 95 ºC for 5-10 min in one cycle .

4.Synthesized cDNA was immediately used as template for qRT-PCR or for long-term storage at -20°C.

2.19.3. Quantitative reverse transcription-PCR (qRT-PCR)

Real time PCR amplification as follows (Goto et al., 2020)

1- Two microliters of cDNA was added to PCR tube.

2- One microliter of each primer was added .

3- The volume was completed to 20µl with DNase-free distilled water.

4- The mixture was mixed well and put in the qPCR machine. The table below reveals the PCR condition

Table 2.3. PCR condition steps

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	95	3min	1
Denaturation	95	15sec	40
Annealing	55	45sec	
Extension	72	60sec	



Figure 2.16. qRT-PCR device

2.19.4. Calculation the fold of gene expression

Levak equation was used to evaluate the fold expression against house keeping gene and control by the following steps (Nikolic et al., 2024)

Ct Control – Ct house keeping control = ΔCt control

Ct sample – Ct house keeping sample =ΔCt sample

Δ Ct sample – Δ Ct control = ΔΔ Ct

Fold of gene expression = (2^{-}ΔΔCt)

Table 2.4. List of primers used for quantitative real-time PCR

Gene	Primer direction	Sequence (5' → 3')	Length (bp)	Annealing Temp (°C)	Product Size (bp) base pairs
Pep	F	GGATGTTTTCCTGAGGCTGT	20	58	59
Pep	R	GGTCAGCTTCCTGGATGAAA	20	58	159
Try	F	TGCAAGGAACCTGAACAATG	20	58	156
Try	R	CCACCAAGACTGAGGGTGTT	20	58	1561
Lip	F	CGCTCCATCCATCTCTTCAT	20	58	133
Lip	R	CCACGTTGTTACAGCGATTC	20	58	133
ACTB	F	CTGTGGCATCCATGAAAC	18	57	88
ACTB	R	CAGACAGCACTGTGTTGG	18	57	88

2.20. Preparation of histological section

Method of preparing tissue sections according to the following steps (Rutland, 2008)

1- Fixation

Place the organs in a 10% formalin solution for 24 hours.

2- Washing

Wash the organs to be cut and examined histologically with regular running water to remove formalin.

3- Dehydration

This process takes approximately two hours, as the samples are passed through ascending concentrations of ethyl alcohol, starting from a concentration of 70% - 80-90-100%, for half an hour at each concentration to rid the samples of water.

4- Clearing

The samples are placed in xylene A solution for a quarter of an hour to get rid of the alcohol, then they are transferred to xylene B solution for a quarter of an hour as well to ensure that the samples are completely clear of alcohol and make the samples transparent and clear.

5- Infiltration

The samples are placed in a mixture of xylene and paraffin wax in a ratio of 1:1 and transferred to an electric oven for a quarter of an hour at a temperature of 60°C. Then the samples are transferred to two stages of molten paraffin for half an hour for each stage.

6- Embedding

The samples are placed in paraffin wax after pouring it into iron molds and left at room temperature until they harden. Then the molds are removed after hardening is complete. Sometimes a hot needle is used in areas where bubbles form near the sample in order to get rid of these bubbles.

7- Trimming sectioning

This process is done using a rotary microtome, which is used mechanically to cut samples with a thickness of 5-7 micrometers. The samples are then transferred to a water bath with a temperature ranging between 37-40°C to brush the cells and prevent their aggregation and accumulation. They are then wiped with Mayer's albumin solution, which is prepared in advance (50 ml egg white with 50 ml glycerin, filtered and added to 1 g of thymol, then kept in the refrigerator until used). The tissue is then placed on a slide with the organ's name and treatment number recorded on it, then transferred to a hot surface at a temperature of 40 for a minute, then the sample is left to dry. The slides are then placed in a xylene solution to get rid of the paraffin wax and passed through decreasing concentrations of ethyl alcohol (100 - 96 - 90 - 70 - 50 - 30%) and finally placed in distilled water.

8- Staining

After removing the samples from the distilled water, they are dried with a dry tissue, then placed in hematoxylin stain for one minute, then washed with water for about a quarter of an hour to distinguish the blue color of the nucleus. After that, eosin alcohol stain is used for 50 seconds to stain the sections red. After that, the sections are washed with 70% alcohol for 5 seconds to highlight the purple color in the cytoplasm.

9- Mounting

Sections are prepared for imaging and microscopic reading. A drop of Candabalsam is placed on the section and allowed to dry at room temperature.

2.21. Statistical analysis

The statistical program Sciences Package Social Statistical (SPSS) version 16 was used to analyze the initial data of the study results, where the Analysis of Variance (ANOVA) test was used, and the level of significant differences between the rates of the criteria included in the current study was extracted by using the least significant differences at the statistical probability level ($P<0.05$).

CHAPTER 3

RESULTS

3.1. Effect of high lipid, protein and bromelain enzyme on body weight of rats

The experimental results showed clear differences in body weight changes between the treatment groups over 30 days. Rats in the control group, fed a standard diet and drinking water ad libitum, showed a moderate weight gain of 37 grams (from 208 grams to 245 grams), equivalent to an average daily gain of 1.23 grams. In contrast, rats fed a high-fat diet showed the largest weight gain, increasing by 75 grams (from 210 grams to 285 grams), with an average daily gain of 2.5 grams. Adding bromelain to the high-fat diet resulted in a significant reduction in weight gain, with animals in this group gaining only 23 grams (0.7 grams/day). Notably, adding bromelain to a high-protein diet resulted in weight loss rather than weight gain: The weight of the rats in this group decreased by 16 grams (from 211 grams to 195 grams), at an average rate of -0.53 grams per day**.**

The results in table 3.1. indicate that while a high-fat diet significantly promotes weight gain, bromelain supplements mitigate this effect and lead to weight loss when combined with a high protein diet**.**

Table 3.1. Effect of high lipid, protein and bromelain enzyme on the body weight of rats

Treatment	Initial body weight, (Mean ± SEM), g	Last day of the experiment body weight (Mean ± SEM),
Control	208.0 ± 4.494^{a}	245.0 ± 1.612^{bc}
Lipid	210.0 ± 1.225^{a}	285.0 ± 1.612^{a}
Protein	209.0 ± 0.894^{a}	261.0 ± 10.807^{b}
Lipid + bromelain	212.0 ± 0.949^{a}	235.0 ± 11.303^{c}
Protein +bromelain	211.0 ± 0.894^{a}	195.0 ± 3.225^{d}
P value	P < 0.102	P < 0.001

a.b, c, d: Values with different superscripts in a line differ significantly, P: probability, $p < 0.01$, SEM: standard error of means

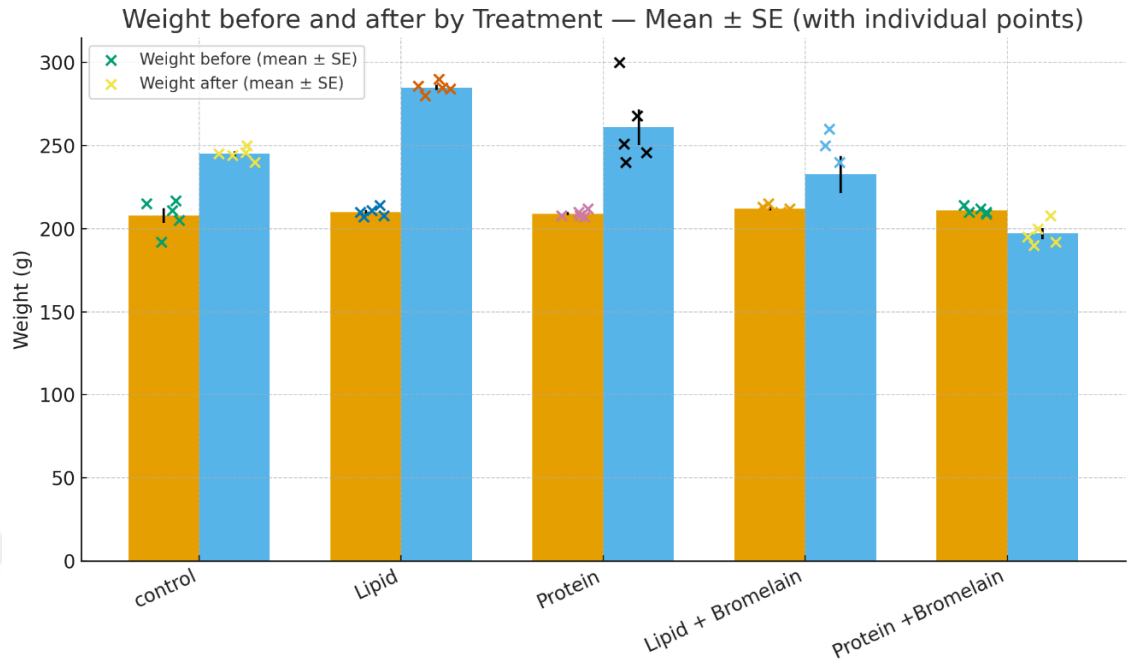


Figure 3.1. Effect of high lipid, protein and bromelain enzyme on body weight of rats

3.2. Effect of high lipid, protein and bromelain enzyme on the liver enzyme levels of rats

The results show that the “Lipid” group showed the highest level of ALP, followed by the “Lipid + Bromelain” and “Protein” groups. The ALP levels in the “Control” and “Protein + Bromelain” groups were significantly lower. This suggests that a high-fat diet may increase ALP levels, while enzymes such as bromelain may have an inhibitory effect. ALT levels were higher in the Lipid and Lipid + Bromelain groups, suggesting a potential negative effect of a high-fat diet on liver health. ALT levels were lower in the Control and Protein + Bromelain groups, possibly suggesting a protective role of bromelain when added to protein. The results show that the highest AST levels were in the “Lipid” group, which may indicate that the high-fat diet caused an increase in liver enzyme activity. The “Control” and “Protein + Bromelain” groups showed lower levels of AST, supporting the conclusion that bromelain reduced liver damage associated with a high-protein or high-fat diet.

Table 3.2. Effect of high lipid, protein and bromelain enzyme on the liver enzyme levels of rats

Treatments	Mean ± SEM AST- GOT, U/L	Mean ± SEM (ALT- GPT) U/L	±Mean SEM (ALP) U/L
Control	41.600 ± 0.92c	31.000±1.22c	135.00 ±2.5d
Lipid	61.800±1.68a	50.200±1.88a	225.400±11.69a
Protein	49.800±0.86b	39.400±0.92b	162.400± 1.63c
lipid+bromelain	52.600±0.92b	49.200±0.66a	191.600±1.77b
Protein+bromelain	40.600±0.92c	32.400±0.74c	139.00±0.70d
P value	P < 0.0001	P < 0.001	P < 0.001

a.b, c, d: Values with different superscripts in a line differ significantly, P: probability, $p < 0.01$, SEM: standard error of means

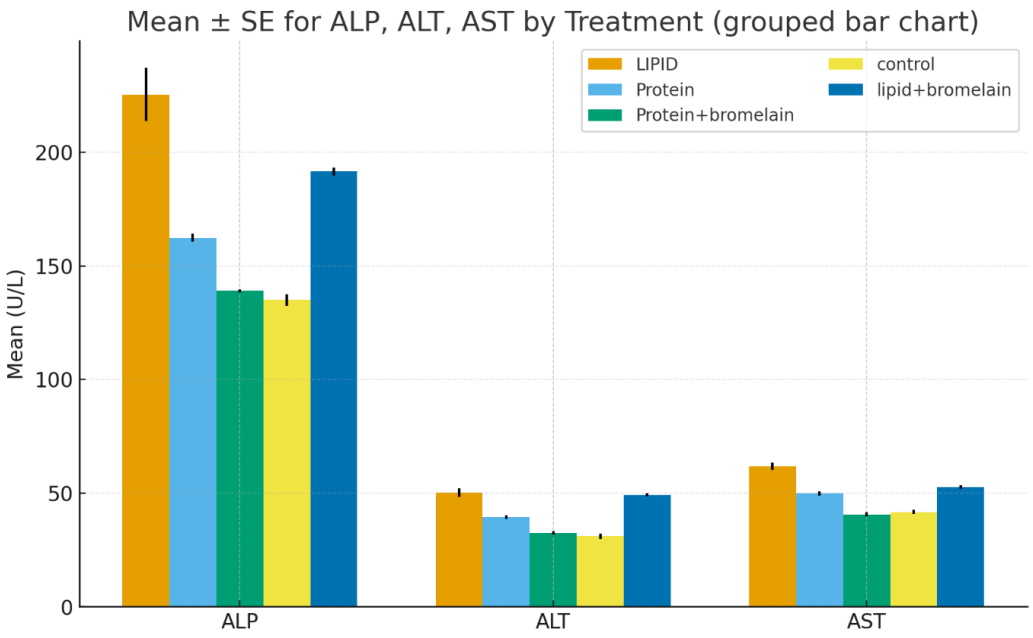


Figure 3.2. Effect of high lipid, protein and bromelain enzyme on the liver enzyme levels of rats

3.3. Effect of high lipid, protein and bromelain enzyme on some whole blood variables in rats

The results showed the control group reflected the normal levels of blood variables in rats without any nutritional or enzymatic intervention compared to the fat group, as this group showed a significant decrease in PLT and a significant increase in RBC, HGB, and WBC compared to the control group. This suggests that a high-fat diet significantly

affects platelet count, decreasing platelet count while increasing red blood cell, hemoglobin, and white blood cell production. These changes may be associated with increased blood viscosity and the risk of thrombosis and inflammation. The protein group showed an increase in PLT compared to control, suggesting that a high-protein diet may enhance platelet formation. We also observed an increase in RBC and HGB, but their values were lower than in the fat group. When bromelain was added to the high-fat diet, we observed a slight increase in PLT compared to the fat-alone group, but it was still lower than the control and protein groups. RBC and HGB also decreased to levels closer to control, suggesting that bromelain may have a protective effect against the negative effects of fat on these hematological variables. WBC values remained elevated, possibly indicating an ongoing immune response. In the protein plus bromelain group, PLT levels were almost identical to the protein alone group, indicating that bromelain does not significantly affect platelets in the presence of the protein. On the other hand, RBC and HGB decreased to levels closer to control, suggesting a balancing effect of bromelain. WBC also decreased to a level lower than the protein alone group and closer to the control group compared to the rest of the groups, which may reflect an anti-inflammatory effect when bromelain was added to the group of animals fed on protein fortified with bromelain enzyme.

Table 3.3. Effect of high lipid, protein and bromelain enzyme on some whole blood variables in rats

Treatments	Mean± SEM WBC, cell/µL	Mean ± SEM HGB, g/dL	Mean ± SEM RBC, cell/µL	Mean ± SEM PLT, cell/µL
Control	9.100±0.360 d	13.22±0.16 c	5.00± 0.11c	208.80±4.11 b
Lipid	15.36±0.273 a	16.42±0.24 a	6.94±0.12 a	152.60±1.83 d
Protein	11.96±0.092 b	15.16±0.12 b	6.06±0.10 b	229.60±4.15 a
Lipid+ bromelain	12.10± 0.16 b	13.42±0.08 c	5.06±0.12 c	181.40±9.37 c
Protein+ bromelain	10.18±0.1 c	13.18±0.08 c	4.96±0.08 c	228.60±5.91 a
P value	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

a.b, c, d: Values with different superscripts in a line differ significantly, P: probability, $p < 0.01$, SEM: standard error of means

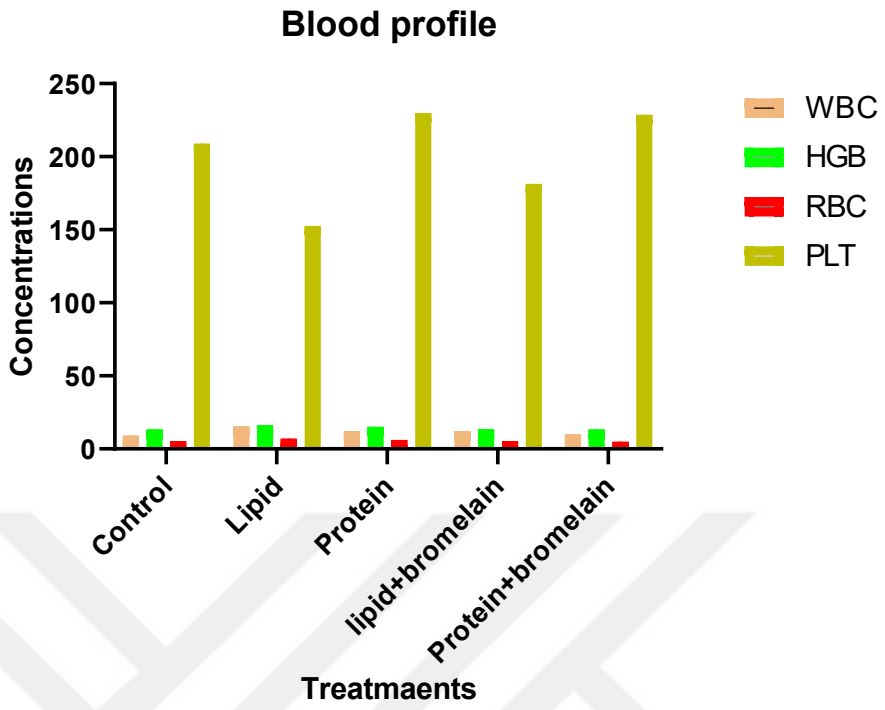


Figure 3.3. Effect of high lipid, protein and bromelain enzyme on some whole blood variables in rats

3.4. Effect of high lipid, protein and bromelain enzyme on lipid profile in serum blood of rats

According to the results in table 3.4 the control group showed moderate levels of lipid levels, which is the basis for comparison with the other groups. The values here indicate a normal level of lipids in the blood without any dietary or enzymatic intervention. In the group that used only fats, we observed a significant increase in the levels of LDL, TG, VLDL, and TC, indicating that a diet rich in fats increases the levels of harmful fats in the blood: LDL (bad cholesterol), which may increase the risk of atherosclerosis, while HDL (good cholesterol), important in metabolic processes and hormones, decreased. In the protein-only group, there was a decrease in VLDL and TG compared to the fat group, with a slight increase in HDL compared to the fat group. This suggests that a high-protein diet may be less harmful to blood lipid levels than a high-fat diet, but it still has a significant effect on LDL levels. In the lipid + bromelain group, it was noted that adding bromelain to the high-fat diet led to a significant decrease in LDL and TC compared to the lipid alone group, with an increase in HDL. This indicates that bromelain may contribute to reducing the harmful effects of fat on blood lipid levels

and improving cholesterol levels. As for the protein group with bromelain, in this group we notice an improvement in all lipid indicators with a decrease in LDL and an increase in HDL, and this indicates that bromelain may enhance the protective effects of a high-protein diet on blood lipid levels.

Table 3.4. Effect of high lipid, protein and bromelain enzyme on lipid profile in serum blood of rats

Treatments	Mean± SEM Total cholesterolmg/dl	Mean ± SEM Glucose mg/dl	Mean ± SEM HDL mg/dl	Mean ± SEM LDL mg/dl	Mean ± SEM VLDL mg/dl
Control	69.80± 2.90^{c}	84.80±3.33b	42.00±1.09b	10.84±3.01c	16.96±0.66b
Lipid	95.60±1.36^{a}	106.80±5.22a	21.20±1.65d	53.04±3.23a	21.36±1.04a
Protein	83.20±3.66b	65.60±4.51c	29.40±0.50c	40.68±2.97b	13.12±0.90c
Lipid+bromelain	73.20±0.86c	84.40±4.38b	45.20±1.59a	11.12±2.42c	16.88±0.87b
Protein+bromelain	73.80±1.28c	84.40±2.54b	48.20±0.86a	8.72±0.70c	16.88±0.50b
P value	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

a, b, c, d: Values with different superscripts in a line differ significantly, P: probability, p < 0.01, SEM: standard error of means, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein

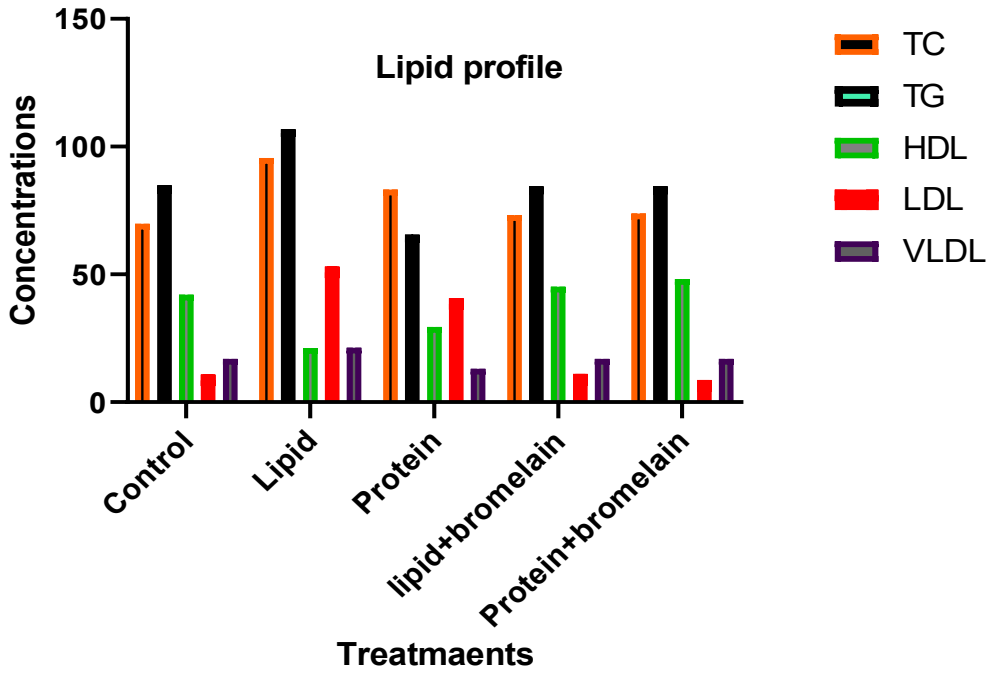


Figure 3.4. Effect of high lipid, protein and bromelain enzyme on lipid profile in serum blood of rats

3.5. Effect of high lipid, protein and bromelain enzyme on urea and creatinine levels in serum blood of rats

The results showed that the high-fat diet led to a significant increase in creatinine and urea levels compared to the control group. This suggests a possible negative effect on kidney function. In the protein group, this group showed a greater increase in creatinine and urea levels compared to the fat and control groups, indicating that a high-protein diet may have a more harmful effect on kidney function, as excess protein may increase the burden on the kidneys, leading to the accumulation of nitrogenous substances such as creatinine and urea in the blood. Adding bromelain to a high-fat diet reduced creatinine and urea levels compared to the fat-only group, indicating that bromelain helped reduce the negative impact of fat on kidney function due to its effective role in breaking down peptide bonds and stimulating enzymes responsible for digesting protein and converting it into smaller molecules that are easier to excrete from the body. In the protein and bromelain group, an improvement in creatinine and urea levels was also observed when bromelain was added to the protein-rich diet. Although the values were still high compared to the control group, they were lower than those observed in the protein alone group, indicating a balancing effect of bromelain when added to the diet.

Table 3.5. Effect of high lipid, protein and bromelain enzyme on urea and creatinine levels in serum blood of rats

Treatments	±Mean SEM Urea mg/dl	Mean ± SEM Creatinine mg/dl
Control	41.20±1.68d	1.10±0.07d
Lipid	52.40±1.07b	1.80±0.11b
Protein	60.20±0.73a	2.20±0.09a
Lipid+bromelain	48.40±0.50c	1.38± 0.08c
Protein+bromelain	49.60±0.50b	1.52± 0.03c
P value	P < 0.001	P < 0.001

a.b, c, d: Values with different superscripts in a line differ significantly, P: probability, $p < 0.01$, SEM: standard error of means

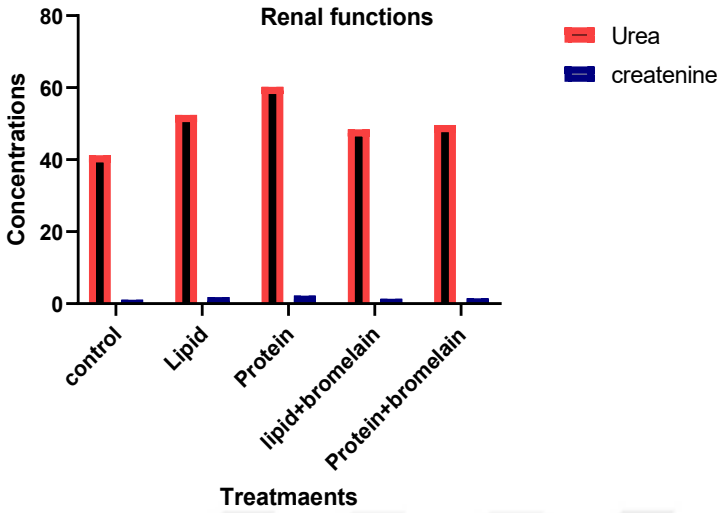


Figure 3.5. Effect of high lipid, protein and bromelain enzyme on urea and creatinine levels in serum blood of rats.

3.6. Effect of high lipid, protein and bromelain enzyme on (MDA) and (GPx) in serum blood of rats

The results showed effect of high lipid, protein and bromelain enzyme on the levels of malondialdehyde (MDA) and glutathione peroxidase (GPx) in rats. The results show that the high-fat diet led to a significant increase in MDA levels, indicating an increase in oxidative stress. In contrast, glutathione peroxidase (GPx) activity was significantly decreased, implying a decrease in the antioxidant capacity of the rats, which may increase the risk of cellular damage. In the protein group, malondialdehyde (MDA) levels were lower than in the fat group, indicating a decrease in oxidative stress, but increased dietary protein still led to some increase in oxidative stress compared to the control group. Glutathione peroxidase (GPx) activity was also decreased, but not to the same levels as in the fat group, suggesting that a high-protein diet has a less severe effect on the antioxidant system. When bromelain was added to the high-fat diet, it reduced malondialdehyde (MDA) levels compared to the fat-alone group, suggesting a protective effect of bromelain in reducing oxidative stress. Glutathione peroxidase (GPx) activity also improved compared to the fat group but was still lower than the control group. In the protein-bromelain group, malondialdehyde (MDA) levels were the lowest among all groups, suggesting that bromelain in the presence of protein may have a powerful effect in reducing oxidative stress. Glutathione peroxidase (GPx) activity

also returned to levels similar to the control group, suggesting an enhancement in antioxidant capacity and a reduction in free radical levels in the body.

Table 3.6. Effect of high lipid, protein and bromelain enzyme on (MDA) and (GPx) in serum blood of rats

Treatments	GPx ±Mean SEM U/mL	MDA Mean ± SEM nmol/mL
Control	2.36±0.09a	10.92±0.16c
Lipid	1.50±0.03c	14.56±0.19a
Protein	1.84±0.05b	11.40±0.23c
Lipid+bromelain	1.88±0.06b	12.86±0.18b
Protein+bromelain	2.38±0.04a	10.16±0.22d
P value	P < 0.0001	P < 0.001

Different letters a – b – c – d indicate significant difference between rates at p < 0.05.

•±indicates standard error level.

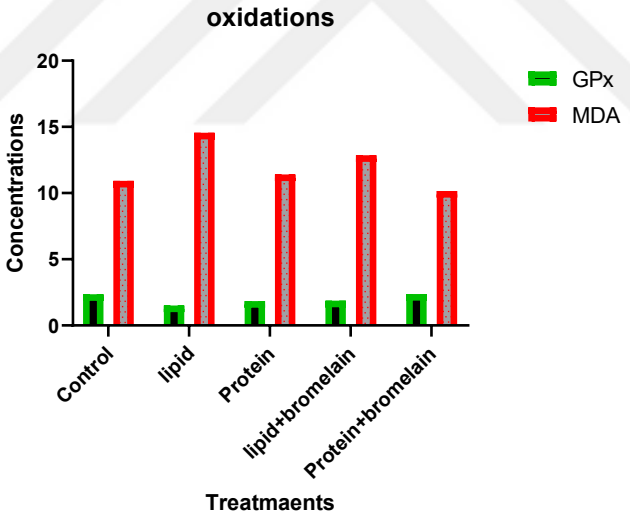


Figure 3.6. Effect of high lipid, protein and bromelain enzyme on (MDA) and (GPx) in serum blood of rats.

3.7. Effect of high lipid, protein and bromelain enzyme on viability of polymorphonuclear in serum blood of rats

The results showed that the quality of nutrition and enzymes had no significant effect on the viability of polymorphonuclear leukocytes (PMNs) in rats, as the PMNs value in the control group was 93.20±0.86, in the high-fat diet (Lipid) group 92.00±0.31, while in the high-protein diet (Protein) group it was 92.00±0.54. As for the group that consumed

fat with the enzyme (Lipid + Bromelain), the PMNs value was 95.20±1.56, and in the group that consumed protein with the enzyme (Protein + Bromelain) the value was 92.80±1.31. Statistical analyses showed no significant differences between the different groups (P > 0.05), indicating that the studied factors (nutrition quality and bromelain enzyme addition) did not significantly affect the viability of PMNs.

Table 3.7. Effect of high lipid, protein and bromelain enzyme on viability of polymorphonuclear in serum blood of rats

Treatments	±Mean SEM PMNs cell/µL
Control	93.20±0.86 a
Lipid	92.00±0.31 a
Protein	92.00±0.54 a
Lipid+bromelain	95.20±1.56 a
Protein+bromelain	92.80±1.31 a
P value	0.15

Different letters a – b – c – d indicate significant difference between rates at $p < 0.05$.

•±indicates standard error level.

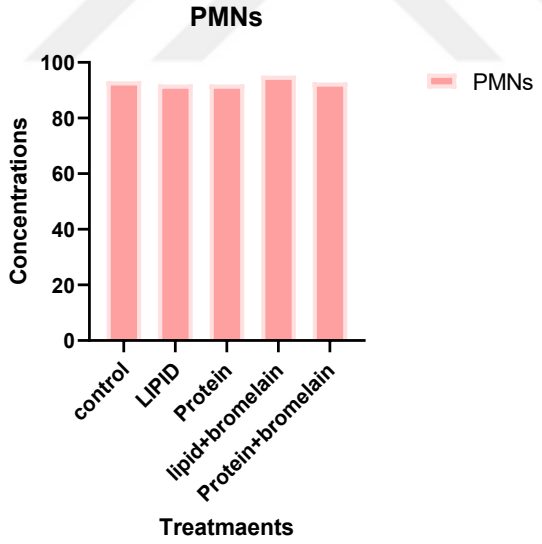


Figure 3.7. Effect of high lipid, protein and bromelain enzyme on viability of polymorphonuclear in serum blood of rats

3.8. Effect of high lipid, protein and bromelain enzyme on phagocytosis in serum blood of rats

The results of the study showed that the quality of nutrition and enzymes significantly affected the levels of phagocytosis in rats. Phagocytosis rates were measured at three

different time points: 90 min, 60 min, and 30 min after exposure to the studied agents. The control group showed the lowest phagocytosis rates with values of 71.20±0.58 at 90 min, 71.60±0.50 at 60 min, and 72.20±1.06 at 30 min. These values were significantly lower than the other groups, as indicated by the letter's "d" at 90 min and "c" at 60 and 30 min. As for the high-fat diet (LIPID) group, phagocytosis rates increased significantly compared to the control group, reaching 82.80±1.01 at 90 minutes, 83.00±0.94 at 60 minutes, and 84.00±0.83 at 30 minutes. The differences were significant, with the letters "c" and "b" to indicate superiority over the control group. The protein-rich feeding group showed the highest phagocytosis rates, which were 90.40±0.87 at 90 minutes, 92.80±1.42 at 60 minutes, and 93.40±1.50 at 30 minutes. These values were significantly higher than those recorded in the previous two groups (LIPID and Control), as shown by the letter “b” at 90 minutes and “a” at 60 and 30 minutes. The group of fats with the enzyme bromelain (Lipid + Bromelain) showed similar results to the protein group, with values reaching 92.60±0.50 at 90 minutes, 94.20±1.15 at 60 minutes, and 95.20±1.56 at 30 minutes. These values were significantly higher than the rest of the groups, as indicated by the letter "a." Finally, the protein + bromelain group showed lower phagocytosis rates than the protein and lipid + bromelain groups, with values of 72.80±1.06 at 90 minutes, 74.00±1.70 at 60 minutes, and 74.00±1.37 at 30 minutes. These values were not significantly different from the control group but lower than the other groups, as shown by the letter "d" at 90 minutes and "c" at 60 and 30 minutes.

Table 3.8. Effect of high lipid, protein and bromelain enzyme on phagocytosis in serum blood of rats

Treatments	±Mean SEM Phago. 30 minutes, %	Mean ± SEM Phago. 60 minutes, %	Mean ± SEM Phago. 90 minutes, %
Control	72.20±1.06 c	71.60±0.50 c	71.20±0.58 d
Lipid	84.00±0.83b	83.00±0.94b	82.80±1.01c
Protein	93.40±1.50a	92.80±1.42a	90.40±0.87b
Lipid+bromelain	95.20±1.56a	94.20±1.15a	92.60±0.50a
Protein+bromelain	74.00± 1.37c	74.00±1.70c	72.80±1.06d
P value	P < 0.0001	P < 0.001	P < 0.001

Different letters a – b – c – d indicate significant difference between rates at p < 0.05.

•±indicates standard error level.

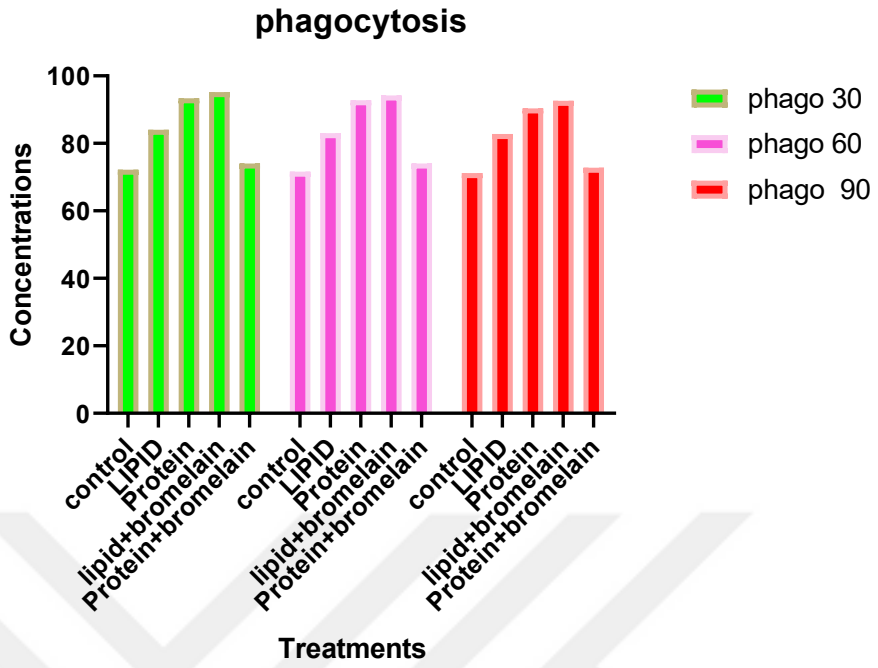


Figure 3.8. Effect of high lipid, protein and bromelain enzyme on phagocytosis in serum blood of rats

3.9. Effect of high lipid, protein and bromelain enzyme on the gene expression of Lipase enzyme in rats

The table 3.1 shows the effect of high lipid, protein and bromelain enzyme on the gene expression of lipase enzyme in rats. The results reflect changes in gene expression levels, which are measured by fold change. Lipid intake significantly increased the gene expression of lipase enzyme, which increased by 149.09 times compared to the control sample. This result indicates that lipid intake alone led to a very large increase in the gene expression of Lipase enzyme, and this increase may be due to lipid stimulation of the digestion and metabolism of fats, which requires increased production of Lipase enzyme to effectively break down fats. In the treatment with protein, protein intake significantly increased the expression of the gene for the enzyme lipase by 22.01 times compared to the control sample. Protein intake also increased the expression of the gene for the enzyme lipase, but at a much lower level compared to lipid. Protein may stimulate the secretion of amino acids necessary for many metabolic processes, including the production of lipase, but its effect is less than that of lipid due to the different nature of metabolism. When bromelain enzyme extracted from pineapple was added to the diet containing fat, the gene expression of lipase enzyme was significantly

enhanced, with a relative change of 427.57 times compared to the control sample. The results showed that the presence of the bromelain enzyme doubled the response of fat cells and led to a significant increase in the production of the lipase enzyme, indicating a cooperative interaction between fat and bromelain in enhancing fat-related metabolic activity. As for the group fed with albumin protein to which bromelain enzyme was added, the results showed a significant decrease in the gene expression of lipase enzyme, with a relative change of only 0.69 times, indicating a decrease in the levels of gene expression of lipase enzyme compared to the control sample.

Table 3.9. Effect of high lipid, protein and bromelain enzyme on the gene expression of Lipase enzyme in rats

Treatments	CT Gene	CT HK	ΔCTE	ΔCTC	ΔΔCt	Fold Change2^-ΔΔCt
Control	26.75	26.75	0	0	0	1
Lipid	26.78666667	31.04333	-4.26	2.96	-7.22	149.0859
Protein	24.62666667	26.21	-1.5	2.96	-4.46	22.00867
Lipid+bromelain	23.64	29.42	-5.78	2.96	-8.74	427.5650147
Protein+bromelain	25.27666667	21.77333333	3.5	2.96	0.54	0.687770909
P value						P < 0.001

Different letters a – b – c – d indicate significant difference between rates at $p < 0.05$.
•±indicates standard error level.
Ct = Cycle threshold
CT HK = Housekeeping gene
CTE= Cycle threshold experiment
ΔCt= Ct gene – Ct Housekeeping gene
ΔΔCt= ΔCt Treatment – ΔCt Control
Fold gene change = $2^{-\Delta\Delta ct}$

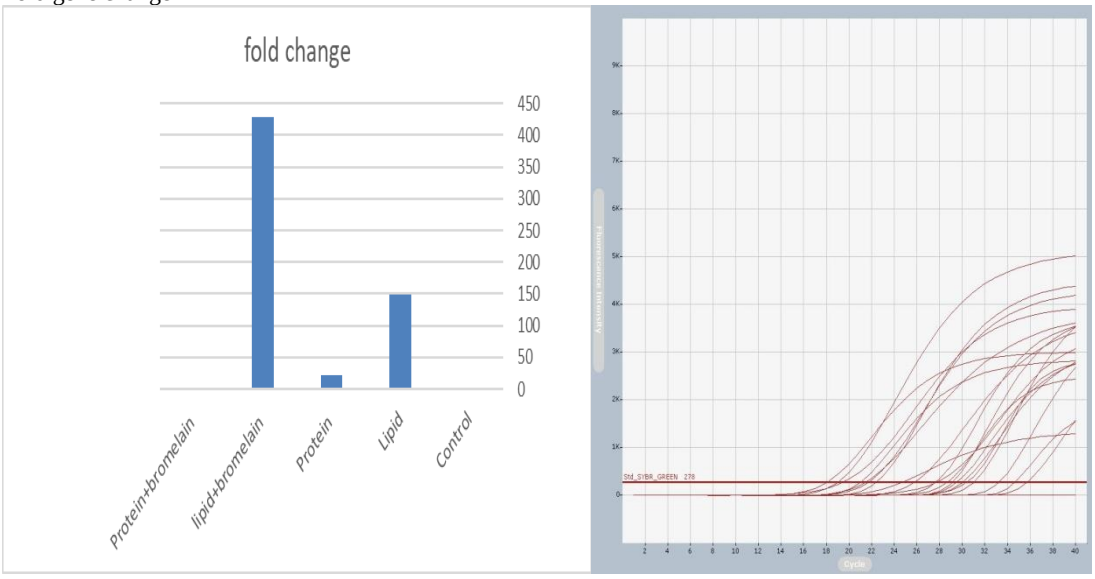


Figure 3.9. Effect of high lipid, protein and bromelain enzyme on lipase gene expression (fold change) in rats.

3.10. Effect of high lipid, protein and bromelain enzyme on pepsin gene expression enzymes in rats

Table 3.10 shows the effect of nutritional quality and bromelain supplementation on pepsin gene expression in rats. The change in gene expression was measured using ΔΔCt- and Fold Change. Fat intake resulted in a very slight increase in pepsin gene expression, with expression increasing by 1.09-fold compared to the control sample. This slight increase suggests a limited effect of fat on the induction of pepsin gene expression. As for the group of rats that were fed a high-protein diet, the results showed a significant increase in the gene expression of the pepsin enzyme, as the relative change reached 15.71 times, as the protein led to stimulating the metabolic pathways that require increased production of digestive enzymes for the protein molecule, such as pepsin. However, when bromelain was added to the diet of the fourth group (fat + bromelain), the gene expression of pepsin increased by 9.49 times, indicating that bromelain enhanced the effect of fat on gene expression but not as strongly as protein alone. When bromelain was added to the diet containing protein, it led to a significant decrease in the gene expression of pepsin, as expression decreased by 0.18 times compared to the control sample.

Table 3.10. Effect of high lipid, protein and bromelain enzyme on pepsin gene expression enzymes in rats

Treatments	CT Gene	CT HK	ΔCTE	ΔCTC	ΔΔCt	Fold Change2^-ΔΔCt
Control	23.39	23.79	0	0	0	1
Lipid	25.68	26.21	-0.53	-0.4	-0.13	1.094293701
Protein	26.67	31.04333333	-4.373333333	-0.4	-3.973333333	15.70697368
Lipid+bromelain	25.77333333	29.42	-3.646666667	-0.4	-3.246666667	9.491701079
Protein+bromelain	23.82333333	21.77333333	2.05	-0.4	2.45	0.183010712
P value						P < 0.001

Different letters a – b – c – d indicate significant difference between rates at $p < 0.05$.

•±indicates standard error level.

Ct = Cycle threshold

CT HK = Housekeeping gene

CTE= Cycle threshold experiment

ΔCt= Ct gene – Ct Housekeeping gene

ΔΔCt= ΔCt Treatment – ΔCt Control

Fold gene change = $2^{-\Delta\Delta ct}$

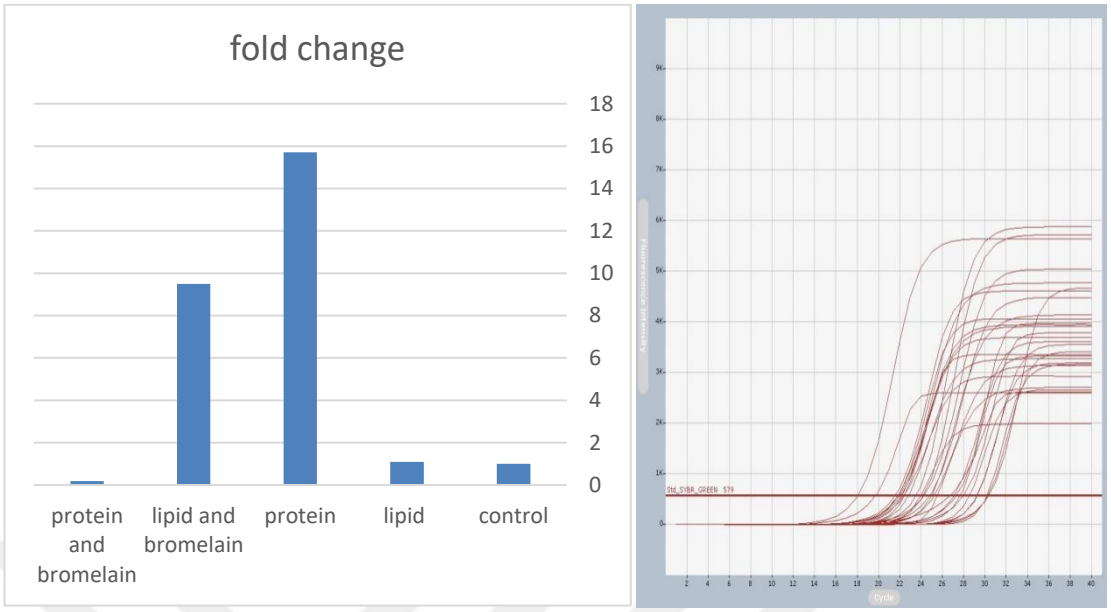


Figure 3.10. Effect of high lipid, protein and bromelain enzyme on Pepsin gene expression enzyme (fold change) in rats.

3.11. Effect of high lipid, protein and bromelain enzyme on gene expression of trypsin enzymes

The results showed the effect of different types of nutrition and bromelain enzyme addition on the gene expression of trypsin enzyme in rats. The values represent the relative fold change in gene expression compared to the control sample. The presence of fat resulted in a slight increase in the gene expression of the enzyme Trypsin by 1.73 times compared to the control sample, indicating a moderate effect of fat in the diet on the stimulation of gene expression of this enzyme. Protein intake led to a very significant increase in the gene expression of the enzyme Trypsin, as expression increased by 65.80 times compared to the control sample. This reflects a strong stimulation of gene expression as a result of protein intake, given the importance of trypsin in protein digestion. When bromelain was added to the group of rats fed fat, the gene expression of the enzyme Trypsin was stimulated by 9.74 times, which indicates that bromelain enhances the effect of fat on gene expression. Adding bromelain to the protein-containing diet significantly decreased the expression of the Trypsin enzyme, with expression decreasing to 0.44 times compared to the control sample.

Table 3.11. Effect of high lipid, protein and bromelain enzyme on gene expression of trypsin enzyme in rats

Treatments	CT Gene	CT HK	ΔCTE	ΔCTC	ΔΔCt	Fold Change2^-ΔΔCt
Control	23.78	23.79	0	0	0	1
Lipid	25.41333	26.21	-0.796666	-0.01	-0.786666	1.725084064
Protein	24.99333	31.0433333	-6.05	-0.01	-6.04	65.79928491
Lipid+bromelain	26.12666	29.42	-3.293333	-0.01	-3.283333	9.736028109
Protein+bromelain	22.93333	21.77333	1.16	-0.01	1.17	0.444421341
P value						P < 0.001

Different letters a – b – c – d indicate significant difference between rates at p < 0.05.

•±indicates standard error level.

Ct = Cycle threshold

CT HK = Housekeeping gene

CTE= Cycle threshold experiment

ΔCt= Ct gene – Ct Housekeeping gene

ΔΔCt= ΔCt Treatment – ΔCt Control

Fold gene change = $2^{-\Delta\Delta ct}$

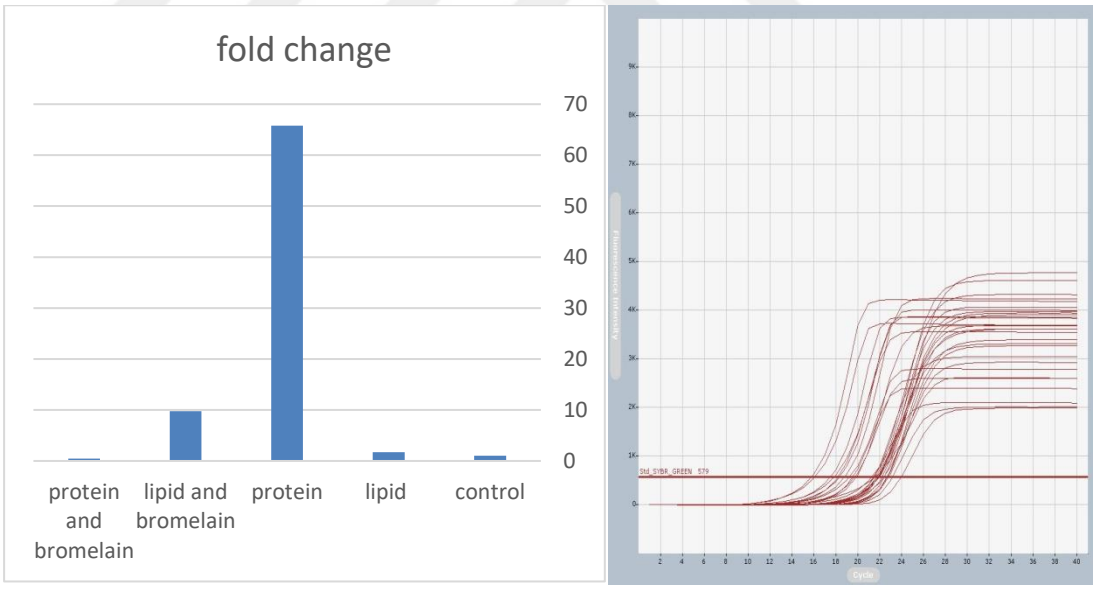


Figure 3.11. Effect of high lipid, protein and bromelain enzyme on gene expression of trypsin enzyme in rats

3.12. Histological examination results

The control group (standard diet + water) showed in the liver of the individuals of the first group a central vein and normal hepatocytes with normal liver sinusoids (*Figures 3.11 & 3.12*). As for the kidney tissue, feeding on a standard diet among the individuals of this group showed normal renal glomeruli with normal renal tubules consisting of the

capillary bundle, Bowman's space, Bowman's capsule, and interstitial tissue with renal tubules (*Figures 3.13 & 3.14*).

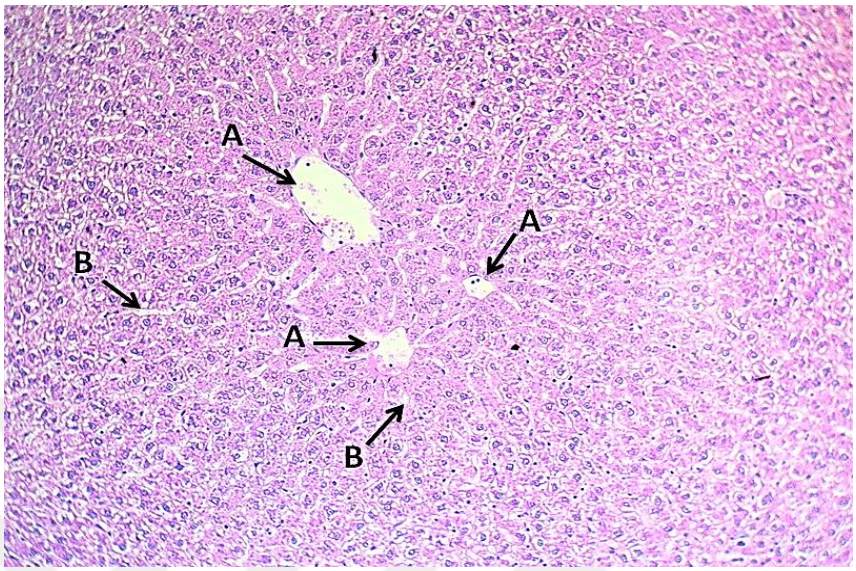


Figure 3.12. Histological section of a rats liver (Group 1) T1

A= central vein
B= hepatocytes
H&E hematoxylin and eosin stain at 100X magnification

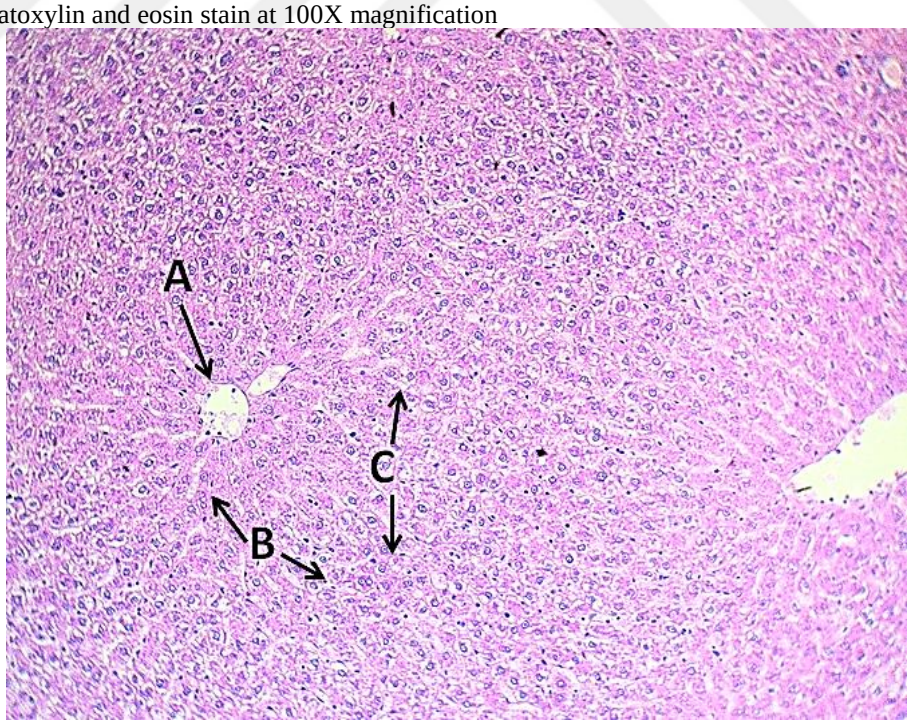


Figure 3.13. Histological section of rats liver (Group 1) T1

A=normal central vein,
B=normal hepatocytes,
C=normal hepatic sinusoids,
H&E hematoxylin and eosin stain at 400X magnification

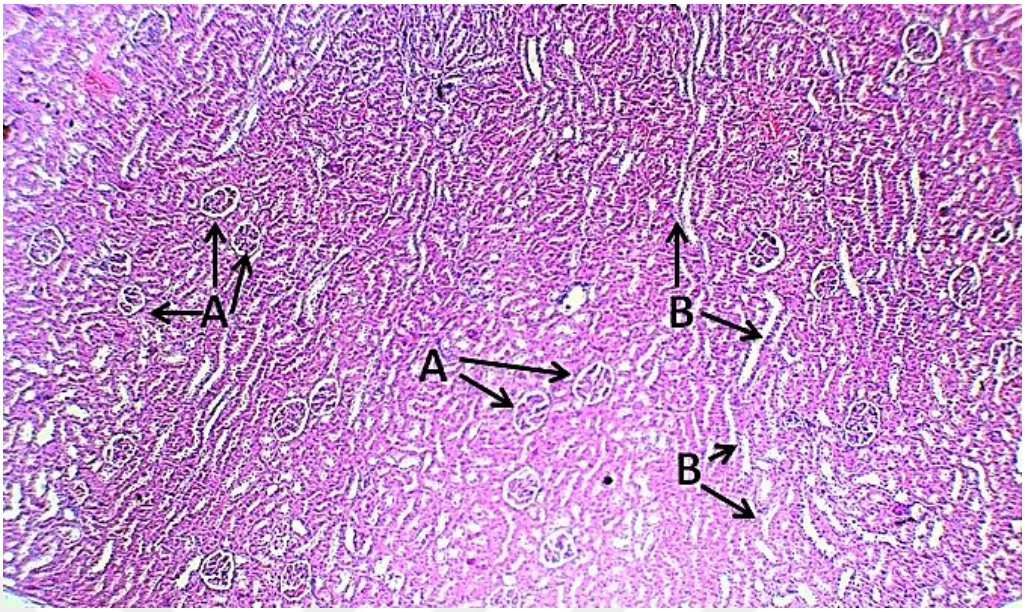


Figure 3.14. Histological section of the kidney of an individual from the first group T1

A = normal renal tubules,
B =normal proximal renal tubules,
H&E hematoxylin and eosin stain at 100X magnification

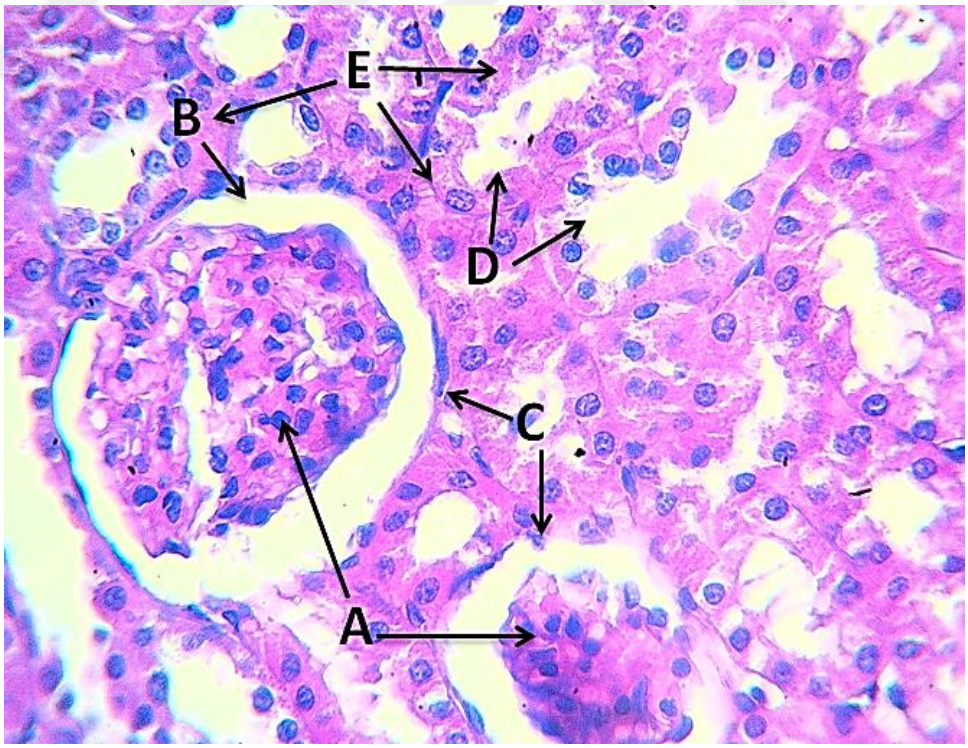


Figure 3.15. Histological section of the kidney of a member of the first group T1

A = normal glomerulus consisting of capillary lumen,
B = Bowman's space, C = Bowman's capsule,
D = normal proximal tubules,
E = interstitial tissue of the kidney.
H&E Hematoxylin and eosin stain at 400X magnification

The second group: (high-fat diet) (4 g of free fat divided into two doses, morning and evening) The results of feeding among the individuals of the second group (high-fat diet + water) showed congestion in the central vein of the liver tissue with fatty degeneration and irregularity of fat cells (*Figure 3.15*). It also showed the presence of large clots in the central vein in the portal area with congestion in the central vein and aggregation of mononuclear inflammatory cells (*Figure 3.16*). Aggregation of lymphocytes in the hepatocytes and acute cellular degeneration and hyperplasia of the bile ducts in the portal area were also observed, in addition to a significant increase in the number of Kopffer cells (*Figure 3.17*). The results of the liver tissue among the individuals of the second group (high-fat) also showed an increase in the number of bile ducts in the portal area with hyperplasia of its epithelium and atherosclerosis of the arteries in the portal area in addition to the explosion of hepatocytes (*Figure 3.18*). As for the kidney tissue among the individuals of this group, the results showed the presence of interstitial hemorrhage between many tissue slices of the kidneys of the individuals of this group, atrophy of the renal glomeruli, and severe star-shaped cellular degeneration (*Figure 3.19*). Also observed in the other kidney tissues of the individuals of this group were a thrombus in the renal vein, fat deposition around the renal vein surrounded by inflammatory cells, in addition to blood congestion in the renal vein, infiltration of lymphocytes in the interstitial tissue, and the presence of hyaline casts in the distal renal tubules (*Figures 3.20. & 3.21*).

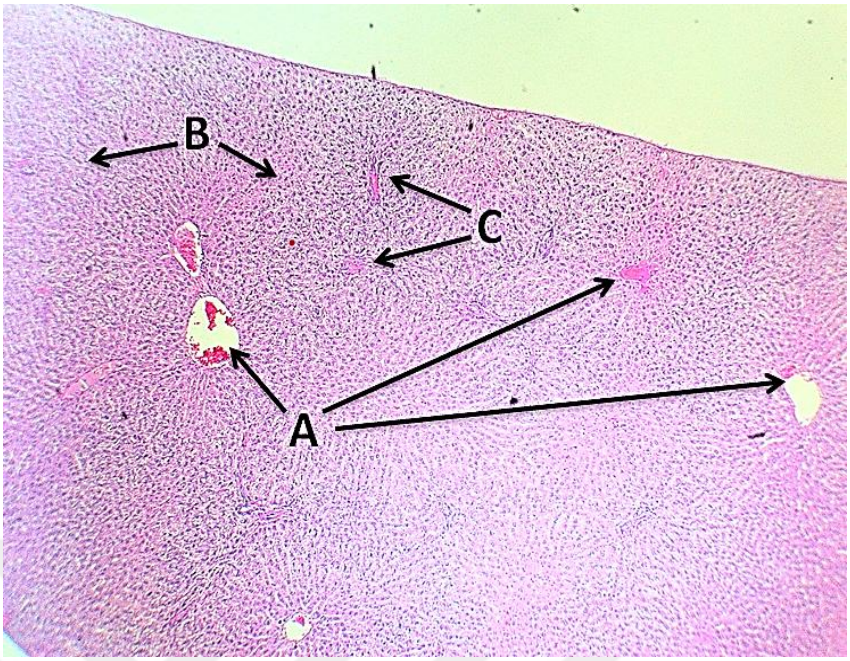


Figure 3.16. Histological section of the liver of one of the individuals in the second group T2

A = Central venous congestion,
B = Fatty degeneration,
C = Disorganization of fat cells.
H&E staining with hematoxylin and eosin at 100X magnification

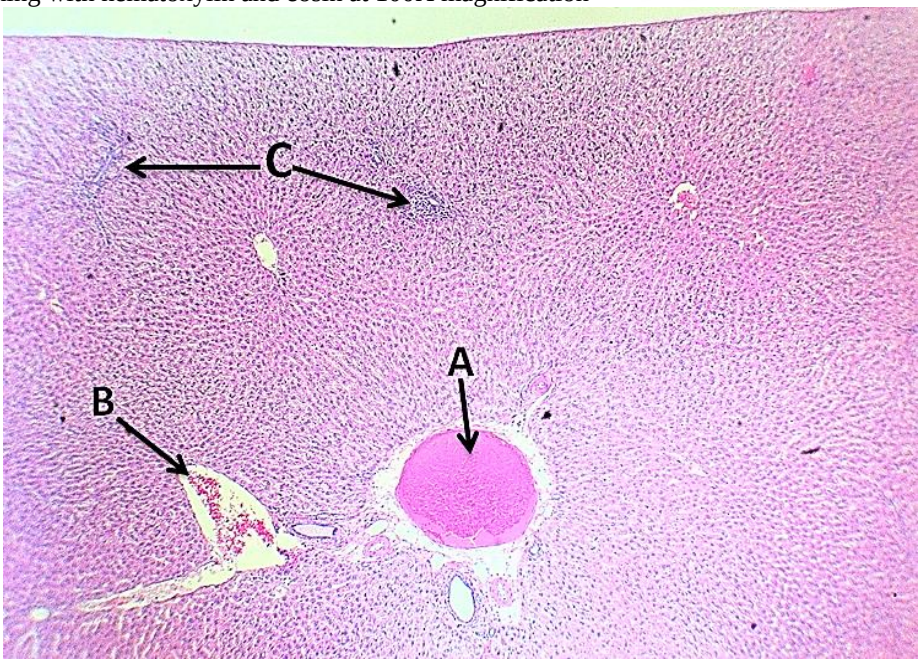


Figure 3.17. Histological section of the liver of a member of the second group T2

A = Large clot in the central vein in the portal area,
B = Central vein congestion,
C = Cluster of mononuclear inflammatory cells.
H&E stain with hematoxylin and eosin at 100X magnification

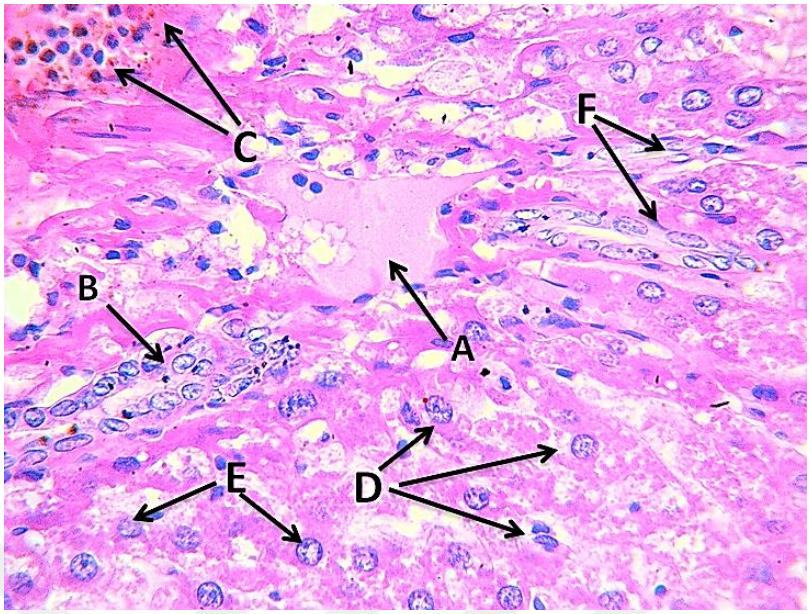


Figure 3.18. Histological section of the liver of a member of the second group T2

A= Edema
B= Hyperplasia of the bile ducts in the portal area,
C= Multicellular and monocellular infiltration,
D= Acute cellular degeneration,
E= Apoptosis,
F= Increased number of Kover cells.
H&E staining with hematoxylin and eosin at 400X magnification

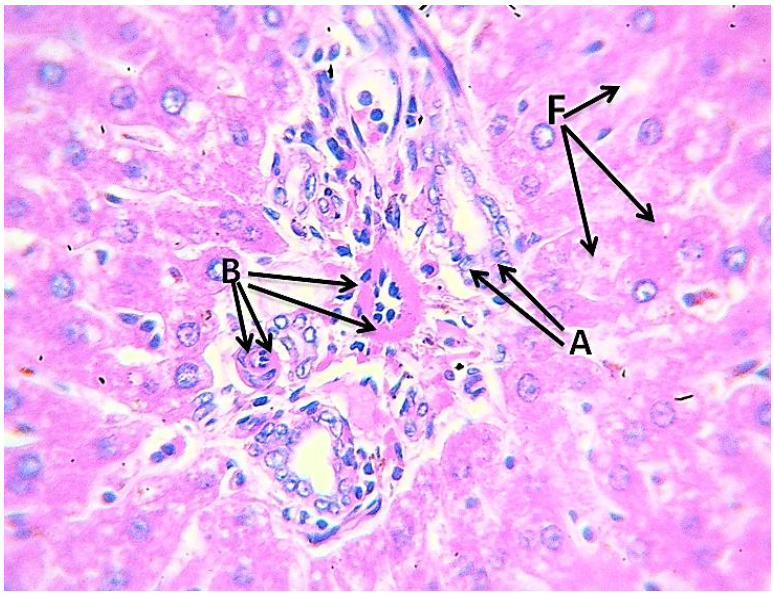


Figure 3.19. Histological section of the liver of a member of the second group T2

A= Increased number of bile ducts in the portal area with hyperplasia of its epithelium,
B= Atherosclerosis of the arteries in the portal area,
C= Hepatocyte rupture with severe cellular degeneration.
H&E staining with hematoxylin and eosin at 400X magnification

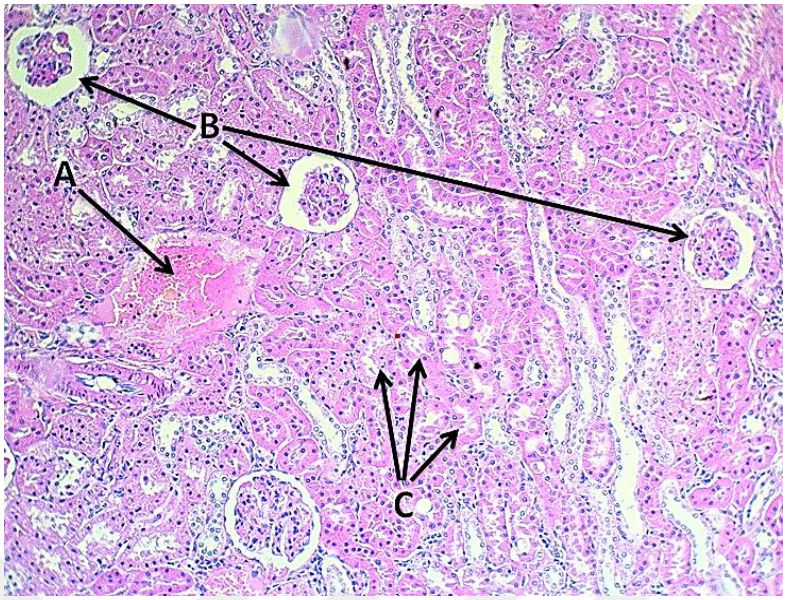


Figure 3.20. Histological section of the kidney of a member of the second group, T2

A = interstitial hemorrhage,
B = renal glomerular atrophy,
C = severe cellular degeneration in the shape of a star.
H&E, hematoxylin and eosin stain, 200X magnification.

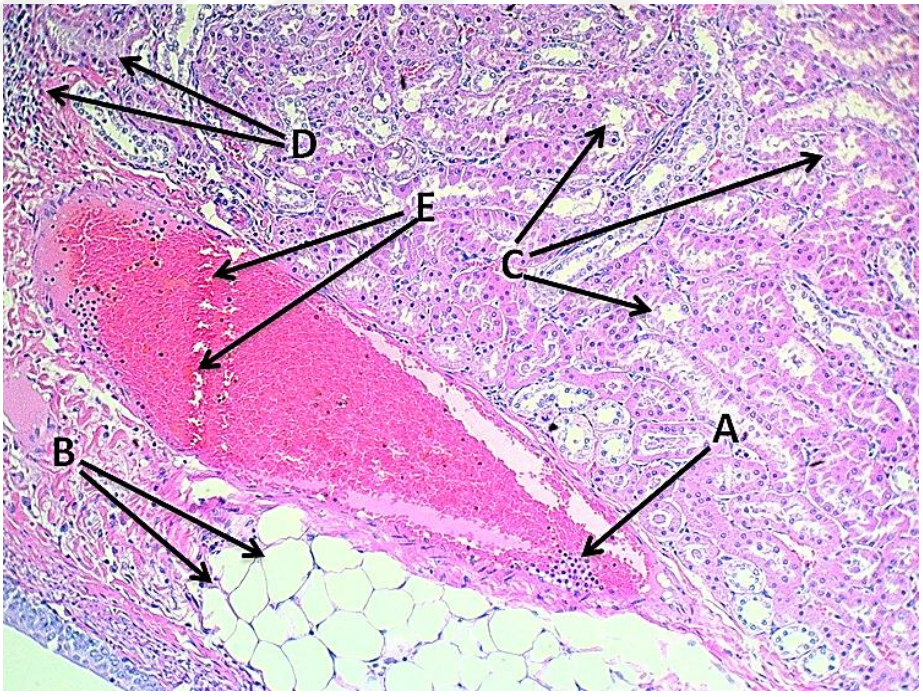


Figure 3.21. Histological section of a kidney from group 2 T2

A = Thrombus in the renal vein
B = Fat deposition around the renal vein surrounded by inflammatory cells,
C = Acute cellular degeneration of the tubules in a star shape,
D = Lymphocyte infiltration in the interstitial tissue,
E = Hematocrit in the renal vein.
H&E staining with hematoxylin and eosin at 200X magnification

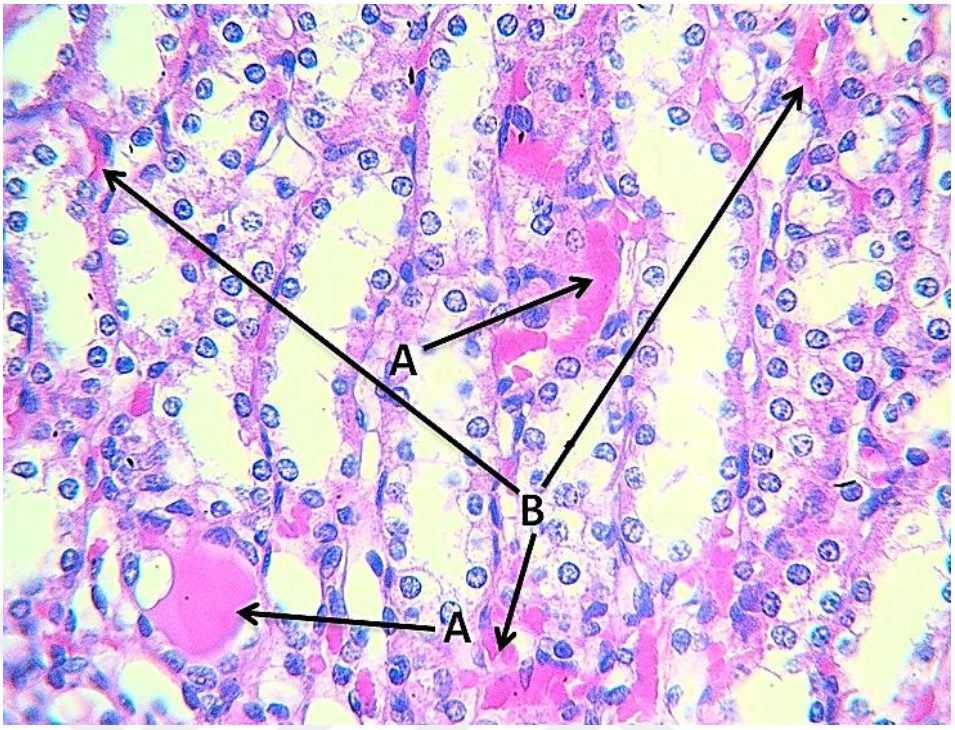


Figure 3.22. Histological section of a kidney from a member of the second group T2

A = Glass blocks in the distal renal tubules,
B=Hemorrhage in the interstitial tissue.
H&E staining with hematoxylin and eosin at 400X magnification

The third group: Albumin group (4 g of albumin divided into two doses daily) showed problems in the cellular tissue of the liver, as there was expansion and congestion in the central vein and hypertrophy of the hepatic cells with narrowing of the sinusoids (*Figure 3.22*). It was also noted that there were clots in the central vein within the liver tissue of individuals in this group, an increase in the number of macrophage cells, necrosis and death of liver cells, in addition to an increase in the effectiveness of programmed death of liver cells (*Figure 3.23*). As for the damage to the renal tissue among the individuals of this group, cystic expansion of the renal glomeruli was observed with an increase in the capillary lumen, in addition to lymphatic infiltration and cavitation around the renal glomeruli, acute cellular degeneration of the renal tubules, and the presence of lymphocyte clusters and adhesions accompanied by acute interstitial hemorrhage and stellate cell necrosis, in addition to high rates of blood clots and congestion (*Figures 3.24 & 3.25 & 2.26*).

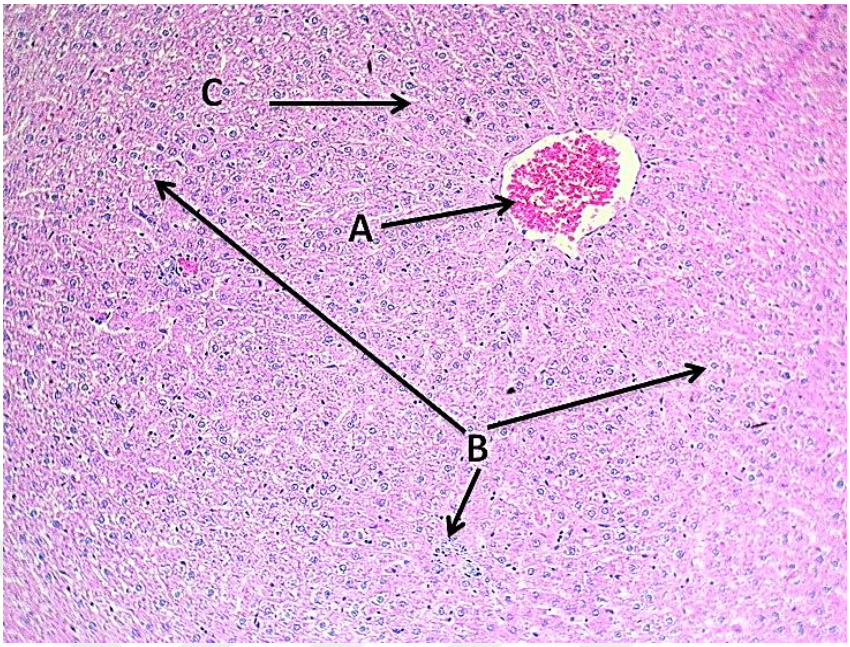


Figure 3.23. Histological section of the liver of a member of the third group T3

A = Dilatation and congestion of the central vein
B = hepatocyte hyperplasia with sinusoidal narrowing
H&E hematoxylin and eosin stain at 100X magnification

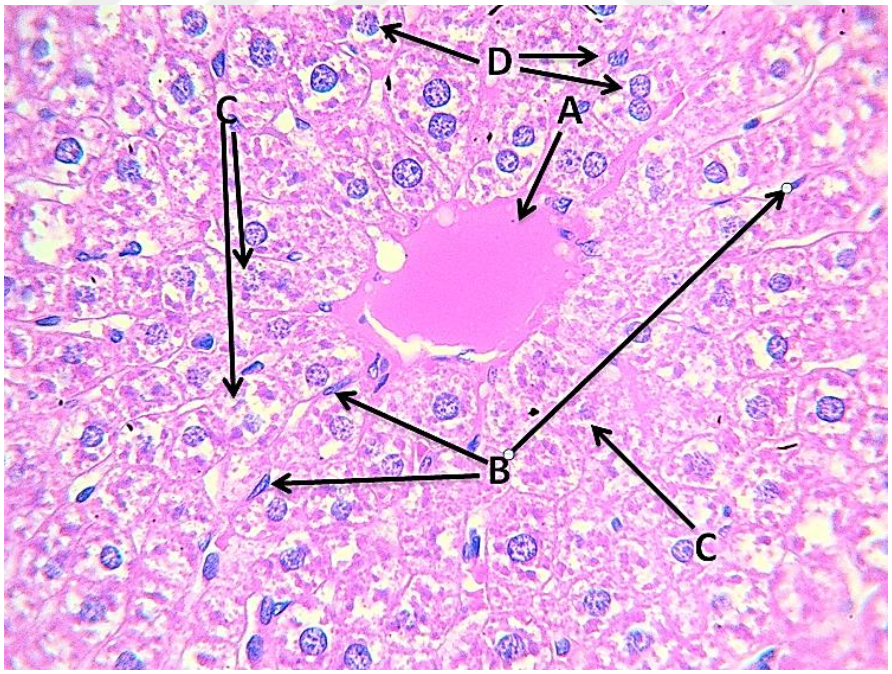


Figure 3.24. Histological section of the liver of a member of the third group T3

A = Central vein thrombosis
B = Increased number of Kopffer cells (macrophages)
C = Necrosis and death of hepatocytes
D = Programmed death of hepatocytes
H&E staining with hematoxylin and eosin at 400X magnification

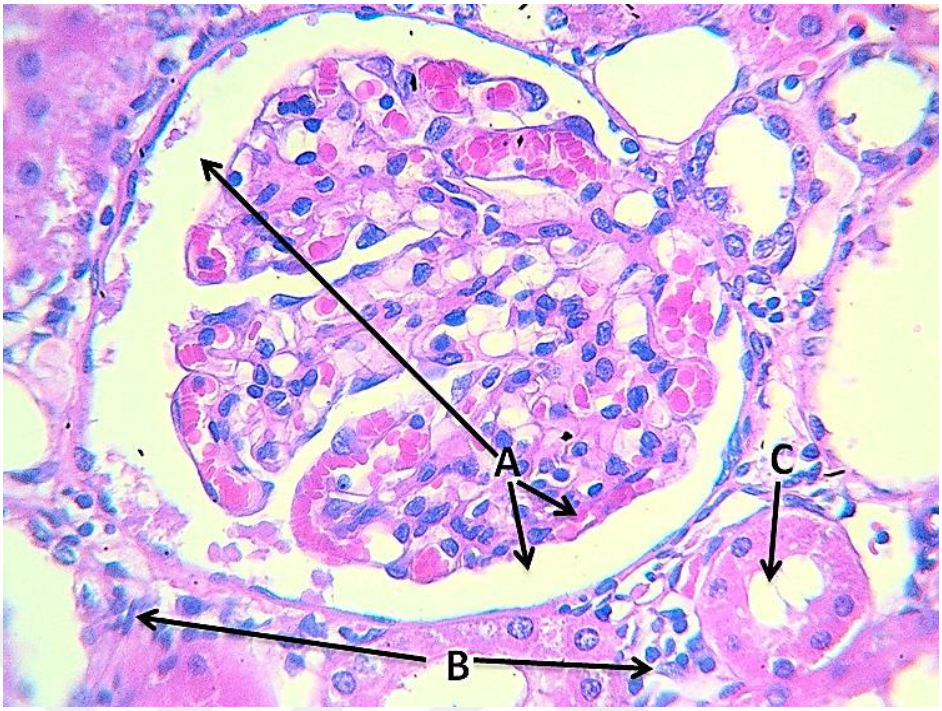


Figure 3.25. Histological section of a kidney from group T3

A = Cystic dilatation of the renal glomeruli with increased capillary
B = Periglomerular lymphocytic infiltration and constriction
C = Acute cellular degeneration of the renal tubules
H&E staining with hematoxylin and eosin at 400X magnification

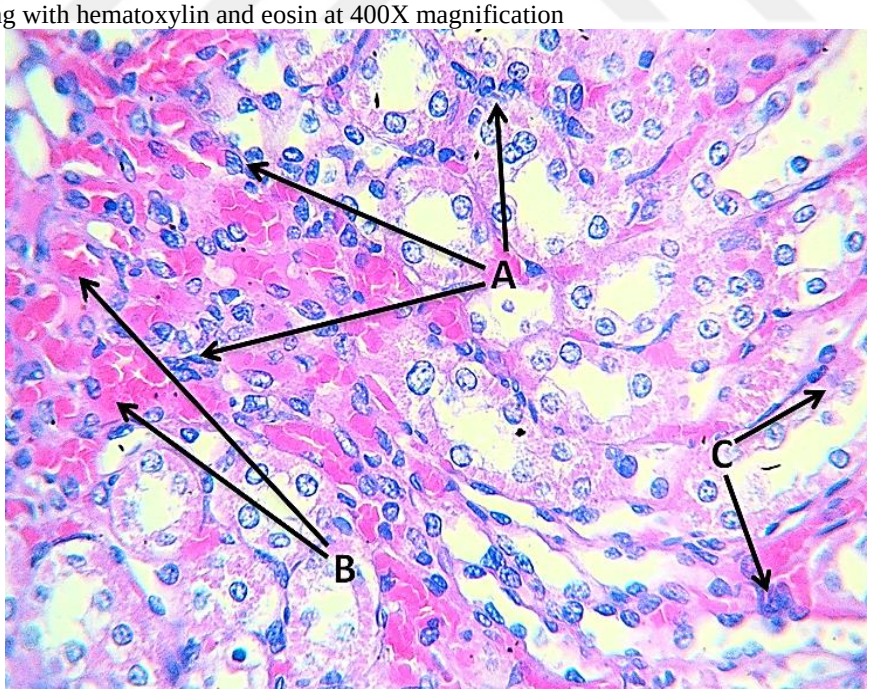


Figure 3.26. Histological section of the kidney of a member of the third group, T3

A = aggregates and adhesions of lymphocytes
B = acute interstitial bleeding
C = cellular degeneration, infiltration, and periglomerular lymphocytosis
H&E hematoxylin and eosin stain at 400X magnification.

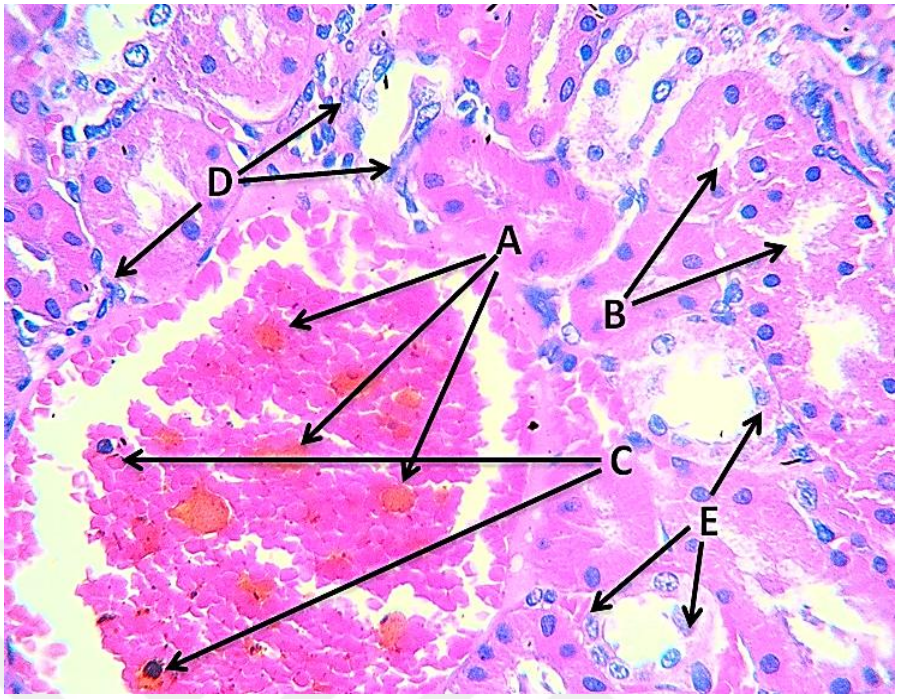


Figure 3.27. Histological section of the kidney of a member of the third group, T3

A = acute interstitial bleeding
B = stellate cell necrosis
C = blood clots and congestion
D = aggregates and adhesions of lymphocytes
E = periglomerular lymphocyte necrosis and necrosis
H&E hematoxylin and eosin stain at 400X magnification

Group 4: Fat + Bromelain (4 mg of bromelain enzyme with fat divided into two doses daily) showed a gradual return to normal tissue when compared with the liver tissue of the individuals in the control group, where the liver cells and bile duct appeared normal (*Figure 3.27*). While other histological examinations among individuals in this group showed slight dilatation and congestion of the central vein and slight rupture of hepatic cells less than what was found among the tissues in the protein and fat group without additives (*Figure 3.28*). However, in the tissues of the remaining individuals, the liver tissue was normal, the central vein appeared normal, and the interstitial tissue was normal (*Figure 3.29*). As for the kidney tissues among the individuals of this group, the glomeruli and renal tubules were normal, as was the case with the lymphocytes, which returned to normal, and there were no cellular infiltrations as was the case with the kidney tissue among the individuals of the control group (*Figure 3.30 & 3.31*).

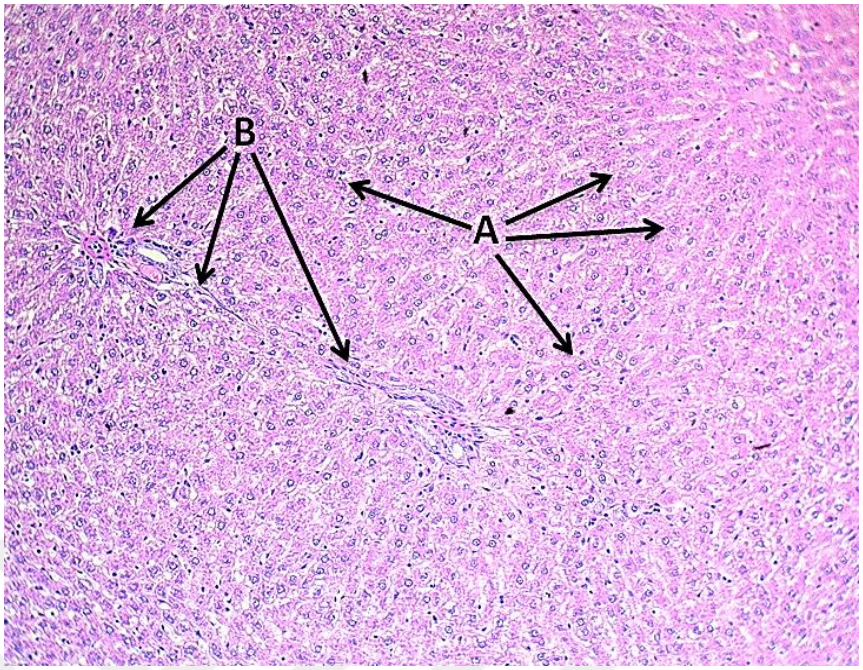


Figure 3.28. Histological section of the liver of a member of the fourth group, T4

A = normal hepatocytes
B = normal bile ducts
H&E hematoxylin and eosin stain at 100X magnification

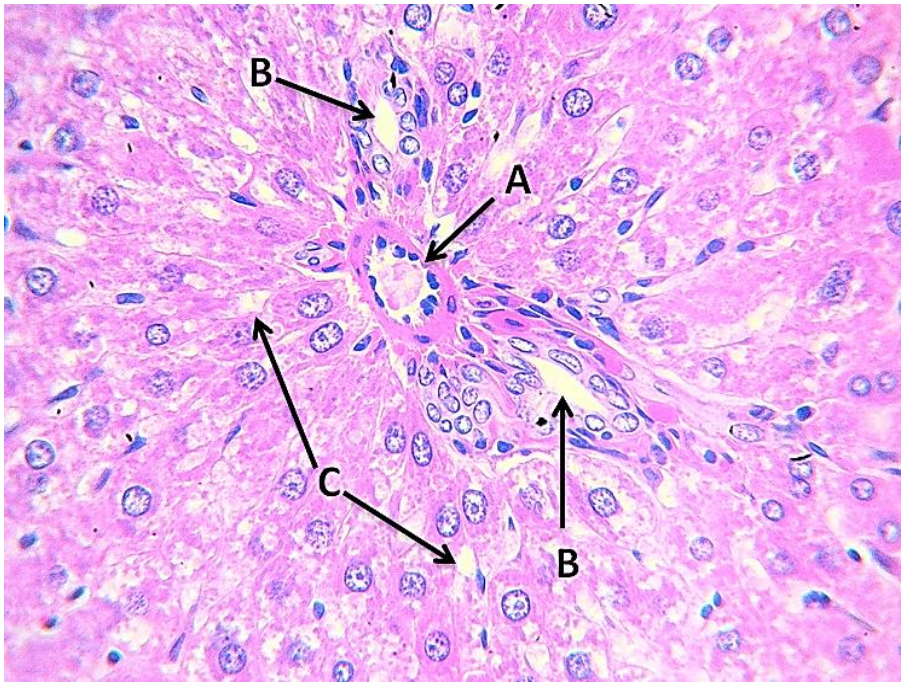


Figure 3.29. Histological section of the liver of a member of the fourth group, T4

A= Slight dilatation and congestion of the central vein
B = normal bile duct
C = slight rupture of hepatocytes
H&E hematoxylin and eosin stain at 400X magnification

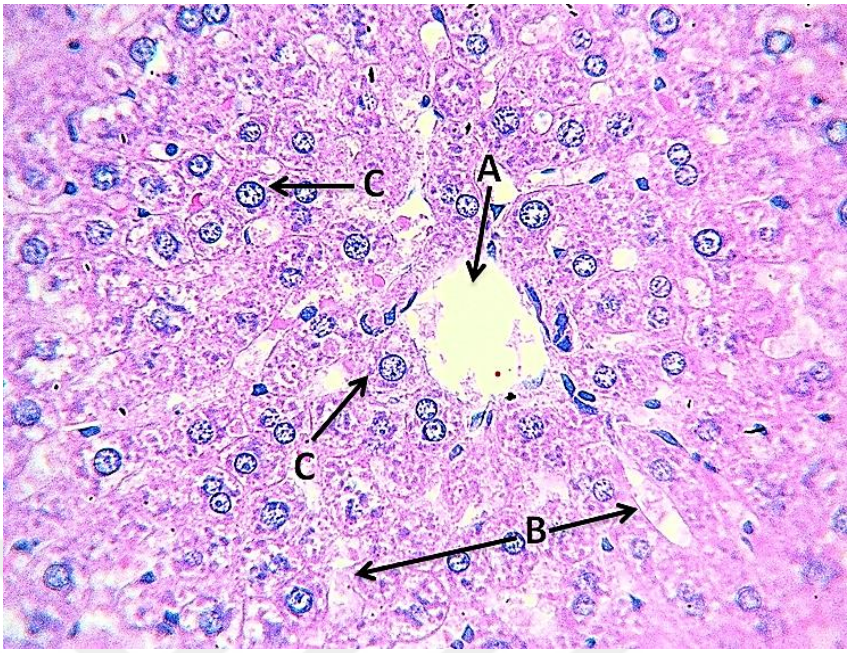


Figure 3.30. Histological section of the liver of a member of the fourth group, T4

A = normal liver tissue with a central vein
B = normal interstitial tissue
C = normal hepatocytes
H&E hematoxylin and eosin stain at 400X magnification

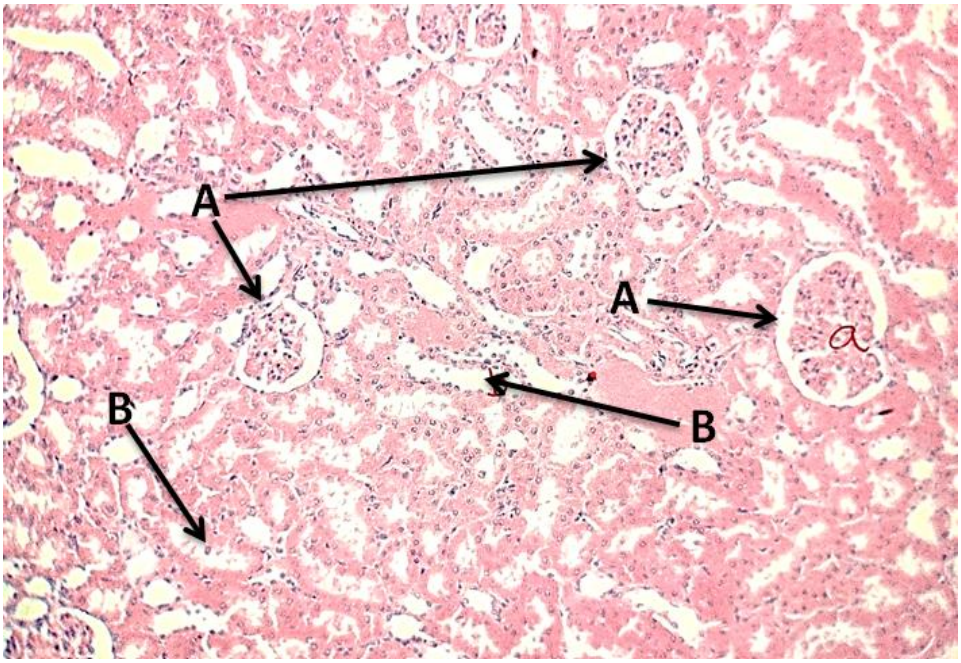


Figure 3.31. Histological section of the kidney of a member of the fourth group, T4

A = normal renal glomerulus
B = normal renal tubules
C = normal lymphocytes with absence of cellular infiltrates
H&E hematoxylin and eosin stain at 100X magnification

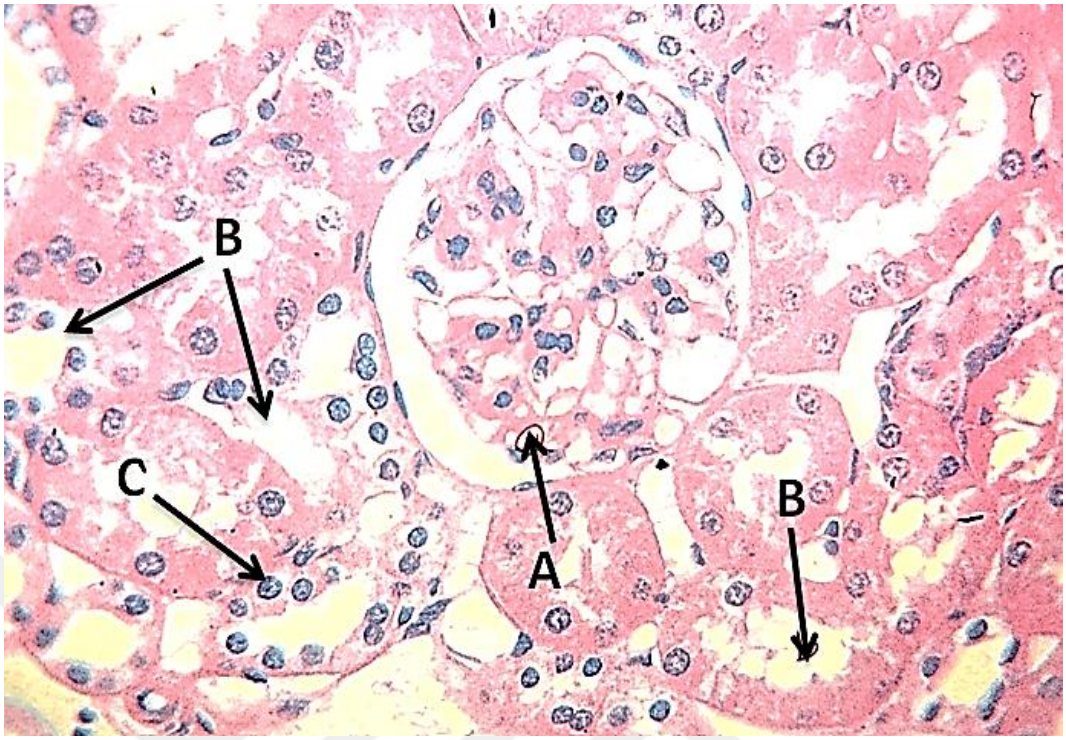


Figure 3.32. Histological section of the kidney of a member of the fourth group, T4

A = normal renal glomerulus
B = normal renal tubules
C = normal lymphocytes with absence of cellular infiltrates
H&E hematoxylin and eosin stain at 400X magnification

Group 5: Albumin + Bromelain enzyme (4 mg of Bromelain enzyme with albumin divided into two doses daily) showed a noticeable return of the liver tissue when compared with the liver tissue of the individuals in the control group, as the liver cells and bile duct appeared normal, while the central vein was free of blood congestion, and the liver sinusoids were normal and equal in size, while the Kupffer cells appeared equal in size and normal as well, indicating the ability of bromelain to accelerate the healing of the liver tissue (*Figures 3.32 & 3.33*). As for the kidney tissue among the individuals of this group, the glomeruli and renal tubules were normal and of consistent size, but slight congestion was observed in the capillary blood vessels, and the lymphocytes appeared of consistent size and distributed normally (*Figure 3.34 & 3.35*).

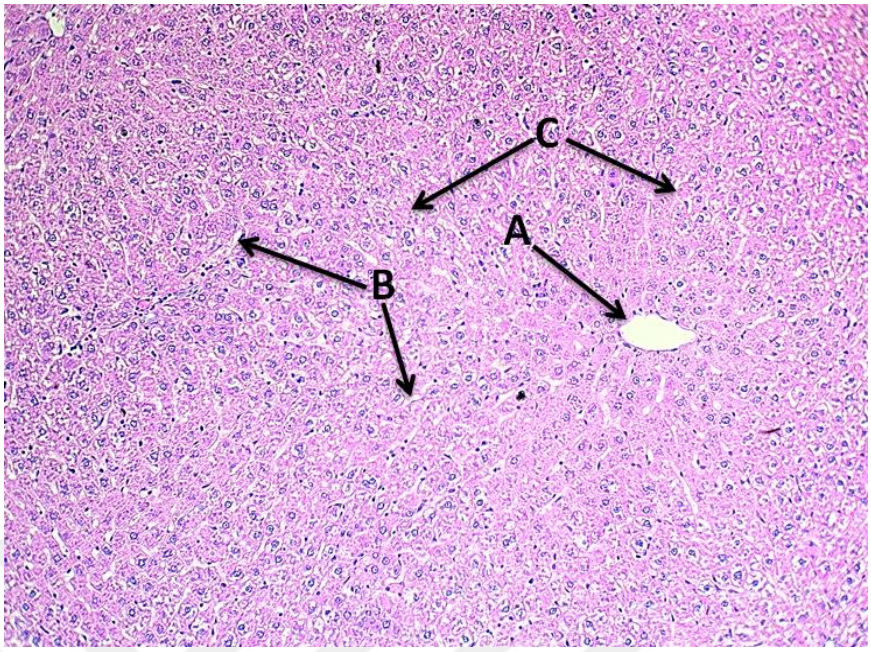


Figure 3.33. Histological section of rats liver (group5) T5

A = normal central vein
B = normal and equal-sized hepatic sinusoids
C = hepatic cells and normal liver tissue
H&E hematoxylin and eosin stain at 100X magnification.

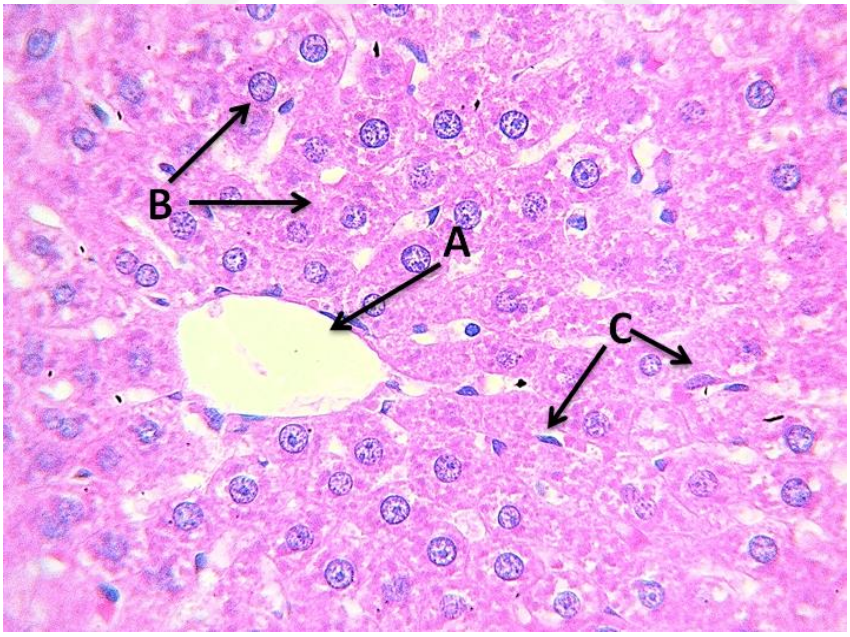


Figure 3.34. Histological section of the liver of a member of the fifth group, T5

A = normal central vein free of blood congestion
B= Hepatocytes and normal liver tissue
C= Kupffer cells are equal in size and normal
H&E hematoxylin and eosin stain at 400X magnification

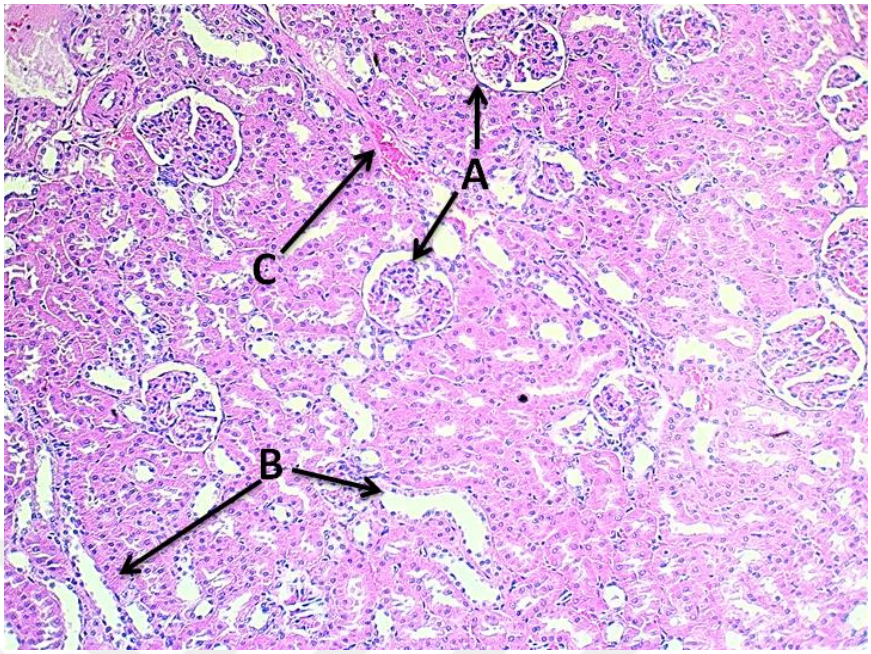


Figure 3.35. Histological section of a kidney from group 5 T5

A= Normal glomeruli
B= Normal sized renal tubules
C= Mild capillary congestion
H&E staining with hematoxylin and eosin at 200X magnification

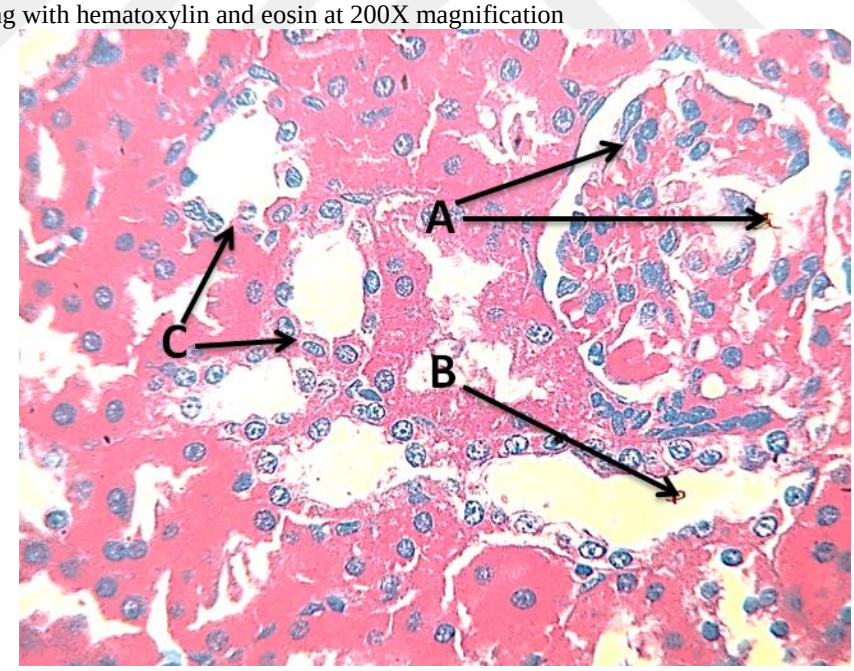


Figure 3.36. Histological section of the kidney of a member of the fifth group, T5

A = normal renal glomeruli
B = normal sized renal tubules
C = uniformly sized cells with normal lymphocytic proliferation
H&E hematoxylin and eosin stain at 400X magnification

CHAPTER 4

DISCUSSION

4.1. Effect of high lipid, protein and bromelain enzyme on body weight of rats

The results showed the weights of the experimental animals increased moderately and were consistent with the amounts of fat, protein, and fiber in the feed prepared for the animals. However, when fat was added at high levels (4 grams of fat per day divided into two doses), this led to a significant increase in weight (Yan et al., 2019). This is due to the high number of calories provided by fat compared to the feed used in the control group. When calories increase, the body stores the excess in fatty tissue, especially around the internal organs. In addition, appetite levels increased in the individuals of this group because the effect of the fat added to the diet on the levels of the satiety hormone (leptin), which is secreted from fat cells, as the efficiency of this hormone responsible for giving the feeling of satiety decreases, which stimulates the mouse to eat more feed and thus gain weight (Miller, 2019). The weight gain among the albumin group (4 grams divided into two daily doses) was also higher than the control group at a rate of 1.7 grams of excess weight per day throughout the study period. This Weight gain is due to the effect of albumin on the balance of hormones that regulate hunger and satiety, in addition to the fact that albumin contains almost all the essential amino acids that the body needs due to its ease of digestion and absorption, which increases the efficiency of converting it into stored energy (Lueders et al., 2022). When adding the enzyme bromelain extracted from pineapple at a rate of 4 mg divided into two doses daily to the diet containing fats, the weight Gain levels were at a rate of 0.7 grams daily compared to the control group. This is due to the effect of bromelain on the absorption of fats in the intestines due to the enzyme's ability to change the composition of fats or their interaction with digestive enzymes in addition to the enzyme's ability to affect intestinal bacteria that play an important role in the digestion process and energy metabolism. On the other hand, the bromelain enzyme also has antioxidant properties.

and reduces inflammation associated with obesity, which reduces the ability of laboratory animals' bodies to store fat (Pereira et al., 2023). When bromelain was added to the diet containing albumin, there was a noticeable decrease in weight among the individuals in the fifth group due to the ability of bromelain to break down proteins, especially albumin, into their original components, which are amino acids, in addition to stimulating metabolism at a faster rate than normal, which caused a high burning of calories. This is what was observed in terms of high activity and, at the same time, a decrease in appetite rates among the individuals in this group. It is also known that increasing the Digestion of proteins by the enzyme bromelain causes the body to provide more ready energy and thus feel full faster, which reduces the amount of food consumed and thus weight loss (Minj and Anand, 2020). In addition, the presence of bromelain on a daily basis in the diet causes the loss of complete proteins consumed with the feed due to its high ability to analyses them and accelerate the digestion process, which hinders the high absorption of food components, which may cause a deficiency in the nutrients necessary for metabolism and cells, which forces the body to use the muscle stores present in its body and analyses them into nutrients necessary for building and repairing damaged tissues, which causes weight loss due to a decrease in muscle mass (Zhang et al., 2021).

4.2. Effect of high lipid, protein and bromelain enzyme on the liver enzyme levels of rats

We find a significant increase in liver enzymes in the group of rats that were fed fats, as a diet rich in fats leads to the formation of fatty liver (Non-Alcoholic Fatty Liver Disease, NAFLD), which is accompanied by an increase in liver fat, which causes severe inflammation and leads to an increase in liver enzymes in the blood (Lian et al., 2020). When bromelain enzyme was added to fats, there was a slight improvement in the levels of liver enzymes in the blood. which indicate liver damage resulting from a high-fat diet, as bromelain enzyme contributes to enhancing liver health and reducing oxidative stress due to its anti-inflammatory properties (Sulumer et al., 2023). As for the albumin protein extracted from eggs, the levels of liver damage were lower due to its content of choline, selenium and vitamin E, which contributed to enhancing liver health (Olgun et al., 2022). Liver health levels increased positively when bromelain was added to the fat-containing diet, approaching normal levels. Liver health also improved, which

was observed. through decreased levels of liver enzymes in the protein-fed group due to the ability of bromelain to break down peptide bonds in proteins and facilitate protein metabolism within living bodies, thus reducing the burden on the liver (Sharma & Vimal, 2023). It also helps improve blood flow and reduce clotting levels resulting from high platelet levels among individuals in this group, and improve blood flow to liver cells, which ensures that oxygen and nutrients reach the liver more effectively. In addition to its role in producing bile acids and contributing to the metabolism of fats and reducing the damage caused by free fat added to the food of experimental animals in this group (Inam-ur-Raheem et al.,2020).

4.3. Effect of high lipid, protein and bromelain enzyme on some whole blood variables in rats

The levels of PLT were low in the rats of the second group. which were fed fats, which caused chronic inflammation that affected the bone marrow and reduced the production of platelets, in addition to the accumulation of platelets in certain areas of the liver and spleen, which caused clots and bleeding in some areas (Kanikarla Marie et al., 2021). This was shown by the histological study of the kidneys and liver. In addition to the damage caused by fats to the liver, which is responsible for regulating the levels of platelets and their production. Fats also caused problems in the blood vessels and arteries and severe damage to the interstitial tissues of the organs, which led to the body draining platelets in an attempt to repair the damage (Yin et al., 2022). This explained the low levels of platelets in individuals in this group and the high levels of white blood cells as an indication of the occurrence of chronic inflammation associated with a high-fat diet (Birulina et al., 2021). As for the high levels of hemoglobin and red blood cells among individuals in this group, this is due to two reasons: The first is the ability of fats to stimulate the bone marrow to produce red blood cells due to their effect on hormonal balance and increased production of the hormone erythropoietin, which stimulates the bone marrow (Piotrowska and Tarnowski, 2021). The second reason is that consuming large amounts of fat can cause problems with digestion and water balance in the body, which may increase the concentration of hemoglobin and red blood cells (Bhadra and Deb, 2020). As for individuals in the protein group, we find an increase in the levels of platelets due to the role of protein in providing all the amino acids that the bone marrow needs to produce platelets (Kuten Pella et al., 2022). On the other hand, proteins may

cause some CRP reactive inflammation, which also stimulates the marrow to produce more platelets in response to reducing inflammatory substances (Sheinenzon et al., 2021). We also find in individuals of this group (protein) a noticeable increase in hemoglobin and red blood cells due to the role of protein that breaks down into amino acids, especially lysine, which is one of the essential amino acids that stimulate the bone marrow to produce red blood cells and hemoglobin, which is four protein chains linked to the iron molecule heme, which is responsible for transporting oxygen from the lungs to the rest of the body (Kosmachevskaya et al., 2021). However, the Results showed an increase in the white blood cell count in the group of rats which fed albumin indicating the presence of an allergic reaction that caused a slight immune response. This is most likely due to the fact that white albumin contains the amino acid methionine, which affects some biochemical processes in the body, including white blood cells (Liu et al., 2023). This is what we found in the swelling of some cellular tissues in the histological anatomy section of this study. However, when bromelain is added to the protein group, we find that platelets return to levels close to normal due to the ability of the enzyme extracted from pineapple (bromelain) to reduce the aggregation and adhesion of platelets to each other (Varilla et al., 2021). This has been proven in many studies on the ability of bromelain as a therapeutic property in preventing the formation of blood clots and reducing levels of inflammation in the body (Prabowo et al., 2021). This also explains the return of white blood cell (WBC) to normal level when the bromelain enzyme is introduced as part of the diet of members of this group.

4.4. Effect of high lipid, protein and bromelain enzyme on lipid profile in serum blood of rats

In the group that used only ghee, we noticed a significant increase in the levels of LDL, TG, VLDL, and TC. This is due to the direct absorption of the consumed fats into the bloodstream and the body's ability to store excess levels of fat in adipose tissue, and the remaining part leaks into the blood and other organs in the body (Everaert et al., 2022). In addition, fat affects the hormones and enzymes that control metabolism, reducing the body's ability to get rid of excess fat, which may cause chronic inflammation in the blood vessels and high levels of VLDL (Very Low-Density Lipoprotein), in addition to high levels of LDL, which pose a threat to the health of the heart and vascular system (Cozzolino et al., 2019). This coincides with a sharp decrease in the concentrations of

high-density lipoprotein (HDL) molecules due to the liver's inability to remove harmful cholesterol due to the decrease in bile concentrations and changes in the composition and structure of HDL, which reduces its effectiveness and concentration in the blood (Stellaard, 2022). As for the protein group, there were lower levels of liver fat compared to the previous group. This is due to the ability of proteins to provide the amino acids necessary for liver health, which allows it to quickly process cell renewal, repair itself, and burn fat faster, in addition to the role of protein in improving insulin sensitivity and enhancing food metabolism (Ko et al., 2020; Papakonstantinou et al., 2022). In the group that was fed fat and supplemented with the bromelain enzyme extracted from pineapple, the levels of total fats and triglycerides were lower than the group that was fed fat alone. This is due to the effective role of the bromelain enzyme in reducing inflammation and helping the liver get rid of the damage resulting from free fats and improving its functions, which is positively reflected in food metabolism (Hikisz and Bernasinska-Slomczewska, 2021; Hu et al., 2022). In the group of rats fed on protein fortified with bromelain enzyme, excellent results were observed in terms of returning blood fats to normal levels. This is due to the ability of bromelain. enzyme to break down proteins, improve digestion, and reduce the burden on the digestive system, and provide amino acids necessary for liver health in renewing its cells and tissues and regulating blood sugar levels by improving insulin sensitivity and preventing the accumulation of fats on the liver and disrupting the mechanism of fatty liver formation, which increases its activity levels and improves metabolism (Liu et al., 2022).

4.5. Effect of high lipid, protein and bromelain enzyme on urea and creatinine levels in serum blood of rats

In the second group, which was fed only fats, there were significant increases in urea and creatinine levels due to the effect of fats on renal cells and the weakening of their efficiency in filtering waste resulting from food metabolism. This is due to the high levels of inflammation in the body and the increase in free radicals resulting from fats, as well as the low levels of blood perfusion and blood flow to the renal tissue due to the high levels of platelets (Ezio, 2022). This is what was observed from clots in the renal tubules shown in the chapter on histology and anatomy in this study. All of these factors caused an increase in urea and creatinine levels, which is the final product of the breakdown of creatinine. In the group of animals that were fed egg protein (albumin),

we notice a sharp increase in the levels of urea and creatinine in the blood. This is due to the nature of the protein as the final product of its metabolism in the body is the production of more urea and creatinine in the blood. When the protein is digested by protease enzymes and broken down into smaller molecules, ammonia is produced, which is classified as a toxic substance for the body. The liver converts the ammonia into urea, which is a less toxic substance, and it is expelled from the body by the kidneys through urine as for creatinine, it results from the breakdown of creatine used to provide energy for the muscles, but the increase in protein levels consumed in food led to an increase in the accumulation of nitrogenous waste, which caused problems in kidney function (Vial et al., 2011; Wybenga et al., 1971). This is what was observed in the damage to the kidney tissue in the histological anatomy section of this study. However, in this study, a vital role was found for the enzyme bromelain in helping the kidneys regain their activity by reducing the burden on them by breaking down the peptide bonds between amino acids and breaking down the protein into smaller and easier units, which causes a decrease in the amount of nitrogen that the kidneys need to process, in addition to the role of the enzyme bromelain in increasing blood flow to the kidney tissue and improving the pumping process towards the glomeruli and renal tubules, thus improving kidney function and also indirectly reducing creatinine levels in the muscles (Liu et al., 2024). Therefore, we note the return of some measures for the individuals in the fat and bromelain groups and the protein and bromelain to levels close to the individuals in the control group in terms of the proportions of urea and creatinine in the blood. It is worth noting that the bromelain enzyme improves the health of the large intestine (colon) by providing the nutrients it needs from amino acids, especially tryptophan, and improving the inner wall of the intestine (inner lining) and increasing the efficiency of the beneficial bacteria present in it, which in turn contributed to raising the levels of protein metabolism and helping the bodies of laboratory animals in reducing the levels of harmful nitrogen in the blood and helping the kidneys improve their performance by producing short-chain fatty acids that are important in reducing kidney inflammation (Lu et al., 2022).

4.6. Effect of high lipid, protein and bromelain enzyme on (MDA) and (GPx) in serum blood of rats

The results show that the high-fat diet led to a significant increase in malondialdehyde (MDA) levels. This is due to the fat being exposed to oxidation, which produces small molecules called free radicals, which cause MDA levels to rise. Low levels of glutathione (GPx) are due to the depletion of the body's store of antioxidants, whose primary function is to convert MDA, hydrogen peroxide and free radicals into less harmful compounds in order to protect cellular tissue (Skrzep-Poloczek et al., 2020). When glutathione levels decrease, this will cause increased oxidative stress, which is indicated by increased levels of malondialdehyde and increased rates of inflammation. This explains the high levels of WBC in individuals in this group as an immune response of the body (Monserrat-Mesquida et al., 2021). As for the members of the protein group, we also find a slight increase in the levels of oxidative stress. This is due to the breakdown of protein into amino acids, which are oxidized and cause the production of more free radicals, but at lower levels than the members of the fat group, because protein enters into the formation and renewal of cells, so the body uses it in higher quantities on an ongoing basis (Chaudhary et al., 2023). High levels of oxidative stress also deplete the body's stores of glutathione, which is a factor that neutralizes cells from damage caused by free radicals (Georgieva et al., 2024). When adding the bromelain enzyme extracted from pineapple to the individuals in the third group (fat + bromelain), we find an increase in glutathione peroxidase levels and a decrease in malondialdehyde compared to the individuals in the fat alone group, which indicates the effective role of the bromelain enzyme in reducing the need for the body’s glutathione stores due to neutralizing the action of free radicals and thus reducing the levels of malondialdehyde (MDA) (Parasuraman et al., 2023). As for the group of animals that were fed a combination of protein and bromelain, the results were different from the individuals of the other groups, as glutathione levels returned to rates close to the control group due to the activity of the bromelain enzyme in raising the value of glutathione GPx. Studies have shown that bromelain enzyme helps activate NF-kB (a key transcription factor), that regulates the cell's response to oxidative stress), which stimulates gene transcription and production of more enzyme, in addition to the role of bromelain in regulating antioxidant genes, as bromelain contributes to increasing the

expression of the NRF2 gene (the gene responsible for producing more antioxidants in the body, including glutathione peroxidase (GPx), in addition to the direct role of the bromelain enzyme as one of the powerful antioxidants that protect cells from oxidative stress, reducing the need to use glutathione GPx as an antioxidant, increasing its levels in the body and increasing its stores (Hikisz and Bernasinska-Slomczewska, 2021).

4.7. Effect of high lipid, protein and bromelain enzyme on viability of polymorphonuclear in serum blood of rats

The results of this experiment showed that all individuals in the groups fed fats, egg protein (albumin) and the combination with the bromelain enzyme did not affect the viability of white blood cells, which indicates that the experiment conducted was safe and did not cause harm to the immune system or weaken it, which increases the chance of the enzyme's success as one of the factors that contribute to enhancing the body's vital activities, raising immunity levels and reducing oxidative stress, which indicates the positivity of the experiment and the nutritional combination used in the various transactions.

4.8. Effect of high lipid, protein and bromelain enzyme on phagocytosis in serum blood of rats

The results showed a clear effect of the treatments used in the experiment on phagocytosis at three different time points calculated on the basis of 90, 60, and 30 minutes after exposure to the treatments studied in the experiment, as the phagocytosis results in the control group were at the lowest. level. This is due to the balanced diet used in the experiment, which contained protein, carbohydrates, and fiber, calculated on the basis of a balance in the animal feed used in the experiment. The individuals in the fat group had significantly higher levels of phagocytosis than the control group (Smith et al., 2015). This is due to the ability of fats to enhance the effectiveness of phagocytosis, either due to their effect on the composition of the cell membrane or the presence of chronic immune response (Calder, 2008). There is another explanation, which is that fats affect the levels of the body's energy balance, leading to weight gain and affecting the function of phagocytosis and the immune system in general (Lee & Dixit, 2020). This was explained by previous readings of blood image values, high levels of inflammation, insulin resistance, and weight gain among the individuals in this

group. This is due to the high fats used in food-stimulating cells to produce larger amounts of cytokines such as tumor necrosis factor (TNF), which causes increased activity of phagocytic cells (Liu et al., 2024). Fats also cause changes in the shape and size of phagocytic cells, making them more effective in phagocytosis. Finally, the accumulation of fats in cellular tissues causes tissue damage which stimulates the inflammatory response and thus raises phagocytic cells and increases phagocytosis among the individuals in this group (Kolb, 2022). Another explanation suggests that fats may alter the gene expression of macrophages and stimulate the body to produce certain proteins that have the ability to increase phagocytosis as a means of adapting to the nutritional status, and stimulating the body to produce receptors that are more sensitive in recognizing foreign bodies (Yan & Horng, 2020). An example of this is that Fats enhance pattern recognition receptors such as PRRs, as these receptors are found on the surface of macrophages and can recognize molecular patterns associated with microbes (PAMPs) or molecular patterns associated with damage (DAMPs) (Li and Wu, 2021). Fats affect the expression and activation of these receptors, and when This type of PRR is activated, and a series of reactions are activated within the cell, leading to the activation of transcription factors, which in turn bind to DNA and lead to an increase or decrease in the target genes (Li & Chang, 2021). In the individuals of the protein group, we notice a high increase in phagocytosis. levels when compared to the individuals of the control and fat groups. This is due to the role of protein in influencing phagocytic cells as it is one of the basic building blocks of cells, including phagocytic cells, in addition to the protein-stimulating cellular signals that affect the activity and inactivity of phagocytic cells such as cytokines, which are small proteins that act as chemical messengers between cells, as proteins contribute to their formation. Increasing their concentration in the cells will enhance the sensitivity of these cells to inflammation in general, even if it is due to a difference in the nutritional balance between proteins and other nutrients (Uribe-Querol and Rosales, 2020). Therefore, we find a significant increase in phagocytosis levels among the individuals of the group of animals that fed on protein when compared to the individuals of the two previous groups (control and fat). When bromelain is added to a diet containing fats, we find a significant increase in the phagocytosis process among individuals in this group. This is due to Bromelain’s ability to improve fat absorption and provide free fatty acids to the body, enhance the construction of the cell membrane of immune cells, and produce cytokines that

stimulate phagocytosis (Chakraborty et al., 2021). When bromelain was introduced into the protein-containing diet of the fifth group, we found that the levels of phagocytosis returned to levels very close to control. This definitely explains the ability of the bromelain enzyme. to digest protein-containing food and return it to its original molecules (amino acids), which helped restore nutritional balance among the members of this group (Hikisz and Bernasinska-Slomczewska, 2021). It is also likely that bromelain destroyed the active sites in the protein and changed the structural composition of the protein, which is known as the denaturation process, and thus the protein lost its ability to interact with the receptors of the immune cells specific to stimulating the phagocytosis process (Ghosh et al., 2021).

4.9. Effect of high lipid, protein and bromelain enzyme on the gene expression of Lipase enzyme in rats

The results showed that increasing fat intake among the individuals in the second group led to an increase in the gene expression of the lipase enzyme, which was measured by fold change, compared to the individuals in the first group (control). This leads us to an important interpretation, which is that increasing fat intake will lead to its absorption in the intestine and its transfer to the blood (Hoseini et al., 2022). This increase will be sensed by the peroxisome proliferator-activated receptors (PPARs), which play a major role in regulating the process of fat metabolism (Montaigne et al., 2021). When fatty acids bind to these receptors, they will activate a series of proteins inside the cell that act as mediators to transmit the signal from the cell surface to the nucleus (Tahri-Joutey et al., 2021). These proteins that were activated by the receptors will combine with nuclear factors, which are precise proteins that bind to specific regions of DNA to begin the process of copying the gene into messenger ribonucleic acid (mRNA), after which the translation process and protein construction will be stimulated and larger quantities of the lipase enzyme, which is responsible for burning fat and converting it into energy (Faber et al., 2022). Increased fat intake causes increased binding of fatty acids to receptors on the surface of cells, transmission of signals to the nucleus, and increased transcription, translation, and expression of the gene responsible for lipase production (Lehner and Quiroga, 2016). It was also noted that the expression Levels of the lipase gene increased among the individuals. in the third group which were fed a high-protein diet , the proteins interfere with the signals reaching the cell nucleus to encode and

translate the lipase enzyme because proteins are broken down into amino acids that also act as cellular signals that stimulate certain pathways, including the fat metabolism pathway (Belinskaia et al., 2020) . In addition, consuming more protein than the body needs will lead to the secretion of more insulin hormone which a responsible for regulating sugar levels and storing fat in the body, also plays a major role in fat metabolism (a fat storage factor), which makes the receptors on the surface of fat cells sense the presence of high amounts of fat, so they send signals to the nucleus about the need to produce more lipase enzyme to digest and metabolize fats and increase the need to extract the latent energy contained within them (Zhang et al., 2022) . In addition, consuming more energy sources such as albumin causes increased fat storage due to its inhibition of the AMPK pathway (a cell energy sensor that increases energy production by burning fats and sugars and is an abbreviation for adenosine monophosphate-activated protein kinase. Inhibiting AMPK reduces the cell's ability to burn fat and encourages its storage, which forces the body to produce lipase as a means of maintaining biological balance and increasing fat digestion to produce energy (Aquilano et al., 2020). When Bromelain was added to the fourth group that was fed fat, the levels of lipase gene expression was very high (427 times compared to the control group). This is due to the ability of bromelain to digest proteins and release amino acids, that act as chemical messengers to the cell nucleus to increase the lipase gene expression. In addition, bromelain has the ability to bind to membrane receptors on the surface of cells, activating them, such as G protein-coupled receptors (GPCRs) (Kumar et al., 2021). Bromelain also has the ability to activate kinases that play an important role in cellular signaling pathways, leading to phosphorylation of transcription factors and increased lipase gene expression (Chen et al., 2022). This indicates cooperative interaction between fat and bromelain in enhancing fat-related metabolic activity. While the levels of gene expression of the lipase enzyme were significantly lower among the individuals of the fifth group that were fed albumin and bromelain enzyme, this is due to the analysis of albumin by the bromelain enzyme into amino acids that are easily absorbed and metabolized, and the effect of bromelain on the structure of chromatin (the complex consisting of DNA and proteins known as histones), which reduced the chances of the lipase enzyme reaching the transcription factors, in addition to the possibility of the presence of negative feedback mechanisms that led to the inhibition of the production of the lipase enzyme when there is an increase in the digestion of

proteins by the bromelain enzyme, which is one of the means used by cells to maintain the balance of enzymes within them, in addition to the increased presence of nutrients in the body, which makes it in a state of sufficiency and does not need to encode genes to produce more digestive enzymes to obtain nutrients due to the lack of need for them (Azarkan et al., 2020).

4.10. Effect of high lipid, protein and bromelain enzyme on pepsin gene expression enzymes in rats

Feeding experimental animals (albino rats) with 4 grams of fat divided into two doses daily led to a very slight increase in the gene expression of the pepsin enzyme, as the expression increased by 1.09 times compared to the control sample. This slight increase indicates a limited effect of fat on stimulating pepsin gene expression due to microRNA (small ribonucleic acid molecules) responsible for regulating gene expression of digestive enzymes), as fat intake did not cause stimulation of cell signals and did not affect the expression of the gene responsible for activating pepsin because the body adjusts enzyme production based on immediate needs, and fat intake will cause an increase in the gene expression of the lipase enzyme responsible for digesting fat molecules and breaking them down into smaller units (Grancieri et al., 2022). Fat intake was not a stimulating factor for the receptors on the surface of cells. responsible for sending cellular signals to the nucleus to start transcription, translation and modification leading to the production of pepsin enzyme (Aquilano et al., 2020). In addition, the presence of fat causes a decrease in stomach acidity (hydrochloric acid), which is also a major reason for reducing pepsin activity, and thus reducing the need to produce more of this enzyme according to the principle of feedback (Li et al., 2022). The presence of high concentrations of inactive pepsin enzyme due to low acidity causes the signals to the nucleus of cells responsible for encoding and producing the gene to stop (Richter et al., 2022). This is due to the loss of the ability of fats to stimulate the production of pepsin enzyme, which is known for its primary function of digesting proteins and not fats. The presence of a simple expression of pepsin enzyme after fat intake is due to the ability of fats to affect stomach hormones such as gastrin, which has a direct effect on the secretion of pepsin enzyme (Santos et al., 2020). In the group of rats fed a high protein diet (4 grams of egg albumin divided into two daily doses), the results showed a significant increase in the gene expression of the pepsin enzyme, as the relative change

reached 15.71 times compared to the control group. This is due to the ability of proteins to stimulate the secretion of gastrin from the stomach cells (Xie et al., 2022). This hormone plays a major role in enhancing the secretion of stomach acids and pepsinogen (the inactive form of pepsin). Therefore, increasing the Protein in the diet stimulates the process of secreting pepsinogen and converting it to active pepsin to increase the stomach's ability to digest consumed proteins (Santos et al., 2020). When meals rich in proteins are eaten, proteins enter the stomach and stimulate special cells known as G-cells located in the gastric mucosa, which secrete the hormone gastrin, which plays several important roles in enhancing digestion. It stimulates parietal cells to secrete hydrochloric acid (HCl), which leads to a decrease in the acidity of the stomach (pH less than 3), which is the main factor in stimulating the conversion of pepsinogen to active pepsin (Geibel, 2022). Gastrin also stimulates chief cells to produce pepsinogen. the inactive form of the enzyme pepsin. The Pepsinogen A gene (PGA) is responsible for the genetic coding of the pepsinogen enzyme (Wölffling, 2020). When gastrin stimulates the gene, an increase in gene transcription occurs in the chief cells, which increases the gene transcription activity of the PGA gene, leading to the production of more mRNA, which is transferred to the ribosomes in the cytoplasm to be translated into the pepsinogen protein, which leads to a high response to the increased gene transcription, so more pepsinogen molecules are produced, which in turn are secreted into the stomach cavity. Due to the acidic environment of the stomach resulting from the secretion of hydrochloric acid, Pepsinogen is converted into active pepsin, so that pepsin acts as a catalyst to break down large proteins present in food into smaller peptides that can be digested and absorbed later in the intestine (Elia et al., 2022). Therefore, the large increase in Gene expression (15.71 fold) demonstrates an adaptive response of the stomach to the increased need for protein-digesting enzymes (Kombat et al., 2024). It is worth mentioning that there is a balance between gastrin, pepsin, and stomach acid, and this balance depends on the feedback mechanism (Geibel, 2022). When proteins are digested, Food turns into chyme and moves to the small intestine to cause a decrease in gastrin levels, which reduces the secretion of pepsinogen and hydrochloric acid (Hyder et al., 2023). This mechanism maintains the balance between the production of digestive enzymes and acids and the body's needs (Moini and Ferdowsi, 2024). In the fourth group, which added the enzyme bromelain extracted from pineapple (4 mg daily divided into two doses), The gene expression of the pepsin

enzyme increased by 9.49 times compared to the control group. This indicates the ability of the enzyme bromelain to affect the ability of fats to stimulate the gene expression of the pepsin enzyme, as It appears that bromelain was a factor that aids digestion in general, in addition to its effect on the receptors found on the surface of stomach cells known as G cells responsible for secreting the hormone gastrin, which, as previously mentioned, plays a major role in stimulating parietal cells to produce basic stomach acid in converting pepsinogen into active pepsin, which increases the demand for pepsin production and vice versa, as explained According to the principle of feedback, stimulating and increasing the transcription of the PGA gene responsible for pepsinogen (Alturki, 2021). While the Gene expression levels of pepsin enzyme were very low when bromelain enzyme was added to the diet of the fifth group that was fed on a high-protein diet, this is due to the ability of the bromelain enzyme to digest protein mainly in the stomach before sending cellular signals to produce pepsin enzyme due to the property of Bromelain as a strong enzyme to digest proteins into small peptides and amino acids, which reduces the need to produce pepsin, causing a sharp decrease in gene expression to 0.18 times compared to the control sample by inhibiting the expression of the PGA gene by affecting transcription factors such as CREB (Cyclic AMP Response Element-Binding Protein) responsible for regulating gene expression within cells (Nirmal et al., 2024).

4.11. Effect of high lipid, protein and bromelain enzyme on gene expression of trypsin enzymes

The results showed a slight increase in the gene expression of the enzyme Trypsin (an enzyme secreted by the pancreas in the small intestine, its main function is to digest proteins into easily absorbed peptides and amino acids), as the gene expression of the trypsin increased by 1.73 times compared to the control sample in the group of animals fed on fats. This indicates a moderate effect of fats in the diet on stimulating the gene expression of this enzyme due to the ability of the fats used among the individuals of this group to stimulate the secretion of the hormone cholecystokinin CCK from the cells of the small intestine (a hormone that stimulates the pancreas to secrete digestive juices such as lipase to digest fats and proteases such as trypsin to digest proteins). Although fats are not a major stimulant for the gene expression of the enzyme trypsin, they stimulate the secretion of the hormone CCK, which acts as a signal that stimulates the

pancreas to secrete trypsin partially, leading to a slight increase in the gene expression of the enzyme Trypsin by 1.73 times compared to the control sample (Cawthon and de La Serre, 2021). With the increase in the presence of fats in the diet increases the secretion of pancreatic juices that contain digestive enzymes, including trypsin, and releases them into the small intestine to enhance the digestion and metabolism process (Vertiprakhov and Ovchinnikova, 2022). In the third group, which was fed albumin (4 grams divided into two doses daily), there was a significant increase in the gene expression of the trypsin enzyme, as the gene expression increased by 65.80 times compared to the control sample. indicating the effective role of the trypsin enzyme in digesting and metabolizing protein. When protein was used in the diet of the rats in this group, the secretion of the CCK hormone from the cells of the small intestine into the blood increased, which acts as a strong cellular signal to the pancreas to copy the genes responsible for producing trypsinogen (the inactive form of the trypsin enzyme), which is activated upon entering the small intestine by enterokinase to transform into active trypsin (Kurz and Seifert, 2021). The higher the concentration of protein consumed in the food, the higher the gene expression of the trypsin enzyme, which increases the body's ability to digest proteins better (Santos et al., 2020). When Bromelain was added to the fourth group of rats that were fed fat; the gene Expression of the enzyme Trypsin was stimulated by 9.74 times. This indicates that bromelain has enhanced the effect of fat on the gene expression of the enzyme Trypsin due to the ability of bromelain extracted from pineapple to increase the sensitivity of the pancreas in receiving cellular signals that ultimately lead to increased transcription and genetic coding for the production of digestive enzymes, including trypsin (Liu et al., 2021). When the bromelain enzyme was added to the protein-containing diet among the individuals. of the fifth group (at a rate of 4 mg per day divided into two doses), this led to a significant decrease in the gene expression of the Trypsin enzyme, as the expression decreased to 0.44 times compared to the control sample. This is Because bromelain reduced the need to secrete trypsin enzyme from the pancreas, as it is also a protein-degrading enzyme, as it caused the analysis of albumin before sending cellular signals to the pancreas to secrete digestive juice containing enzymes, including trypsin (Yu et al., 2022; Jahandideh & Wu, 2021). This affected the response of intestinal and pancreatic cells to CCK signals, which were observed to increase with protein intake. However, adding bromelain reduced these signals, which led to a decrease in trypsin gene expression

(Santos et al., 2020). In addition, the body naturally uses the negative feedback theory in the process of regulating the production of digestive enzymes. When there are sufficient concentrations of enzymes specialized in digesting proteins, as is the case with bromelain, there is no strong need to produce other protein-digesting enzymes. Therefore, the genetic coding for the trypsin enzyme decreases when bromelain is added to a diet containing protein (Kansakar et al., 2024).

4.12. Histological study

4.12.1. Effect of treatments on liver and kidney tissues in rats

The results of the experiment showed that the diet of the individuals in the first group did not cause any tissue damage, and the signs were clear from the diseases among the individuals in this group due to its balanced content of nutrients. The high-fat diet added to the standard diet and drinking water among the individuals of the Second group T2 recorded health problems and tissue damage represented by fatty degeneration with the formation of cavities within the liver tissue, in addition to blood congestion in the central veins, clots, and cell infiltrations and the spread of lymphocytes indicating acute inflammation with the activation of the programmed cell death property (Saleh et al., 2024). At that time, the kidney tissue showed cellular degeneration and fat deposition around the renal glomeruli with cellular infiltration and the appearance of glass moulds in the renal tubules with bleeding (Maletha, 2023). Triglycerides are a natural source in the blood as they are an important source of energy, but when their levels rise due to their increased concentration in food, it leads to serious complications, causing fatty liver disease and the inability of this important organ to filter and purify the blood from toxins and waste resulting from metabolism, so inflammatory causes increase and lead to organic dysfunction in many other organs within the body, in addition to the appearance of cavities in the kidney and liver and the spread of inflammatory cells and free radicals in the body Reactive oxygen species causing oxidative stress to the cells with Apoptosis occurs in an attempt by the body's immune system to fight infection and tissue damage, in addition necrosis and clots in blood vessels (Wu et al., 2020). As for the high-protein diet (albumin), prescribed to the individuals of the third group T3, which included protein food with standard fodder and drinking water as prescribed for the rest of the individuals of the other groups, there were congestions and expansions in

the central veins, increased swelling and cavities of the liver cells, and an increase in the number of inflammatory cells (Kupffer cells) with an increase in the programmed death property of the liver cells (Kumar et al., 2021). The results of the kidney tissue showed a noticeable and wide expansion of the renal glomeruli with severe cellular degeneration of the tubules and lymphatic infiltration around the renal glomeruli in addition to severe bleeding in different areas of the tissue. Proteins accelerate muscle growth by providing essential amino acids. for rapid and effective construction (Harkema et al., 2016). They are involved in building all cells in the body, but excessive protein in the diet has health risks such as weight gain, problems with metabolism and gene expression for construction and demolition as the excess is converted into glucose due to high levels of insulin and stored as fat (Matsuoka et al., 2017; Ruan et al., 2018). In addition, high levels of protein can strain the kidneys and liver due to increased levels of urea and creatinine in the body and the body's attempt to get rid of these wastes by raising urine rates, which may cause cellular dehydration (Al-Mohammadi et al., 2020). High protein intake in food causes increased oxidative stress due to the conversion of excess proteins into fats spread among the body tissues, which increases blood viscosity and impedes blood flow to organs, especially those containing capillaries such as the kidneys, which are full of renal tubules with narrow diameters, which may cause blood clots or bleeding in some cases due to increased rates of cellular infiltration (Thomas, 2022). As well as increased rates of free radicals that cause liver fibrosis and increased cell divisions due to the imbalance between the cell components of nuclei and organelles (Rives et al., 2020). It also causes increased kidney fatigue which due to high urea levels, causing enlargement of the renal glomeruli with damage to the tubules accompanying acute cellular degeneration (Chanvillard et al., 2022). The diet containing the standard feed, accompanied by a high-fat diet but enhanced with The bromelain enzyme extracted from pineapple had very slight changes in the tissues when compared with the normal tissues of the individuals in the first group (standard feed), as the tissues were almost normal due to the ability of this enzyme to improve the digestion process and food metabolism and is an enhancing factor in treating inflammation and wound healing, muscle strain, and arthritis by preventing inflammation and its ability to breakdown fibrin, which forms clots and blood clots, in addition to its ability to break down kainite, which is one of the chains of inflammatory processes that cause inflammatory cells to be drawn to the sites of inflammation (Kumar et al., 2023). As bromelain prevents pain

by reducing inflammation and shortening the recovery period, in addition to the ability of the bromelain enzyme to prevent free radicals and reduce oxidative stress clearly, which is classified as the main cause of cell damage and programmed death (Bottega et al., 2021; Pavan et al., 2012). The results of the fifth group, which was prescribed a diet consisting of standard feed and high-protein diet food (egg albumin) fortified with bromelain enzyme, showed that the liver and Kidney tissues were normal and clearly free of pathological and tissue lesions. This is due to the ability of the bromelain enzyme extracted from pineapple to clearly reduce inflammation and prevent neutrophil cells from migrating through the blood to the sites of inflammation, causing pain and cramps (Pham-Huy and Huy, 2022). The bromelain enzyme also activates the immune system and reduces edema, in addition to its role in breaking down fibrin through its similar action to plasminogen found in the walls of blood vessels, as fibrin breaks down and the process of blood clot formation and the accompanying health problems represented by atrophy, cell Degeneration and programmed death stop as the bromelain enzyme works to break down protein materials into their basic components to facilitate their absorption by lymphocytes and the digestive system again and use them as sources of energy (Balakireva et al., 2019).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

High-fats diets brought about weight benefit and expanded liver enzymes (GOT GPT, and ALP) with the increase of hepatic steatosis. The take a look at validated that nutritional (fats and protein) notably impacts the gene expression of digestive enzymes (lipase, pepsin, and trypsin) in rats, confirming the interplay between food plan and molecular regulation. Bromelain mitigated these effects and enhanced the following:

1- Restoration of metabolic, enzymatic, and hormonal balance according to feedback regulation principles.

2- Contribution to weight regulation in experimental rats, counteracting the excessive gain induced by high-fat and high-protein diets.

3- Recovery of renal and hepatic tissues.

4- Improvement of digestive processes, enhancing nutrient absorption and overall health.

5- Enhancement of immunity and reduction of pro-inflammatory factors.

6- Reduction of oxidative stress, contributing to long-term cancer prevention.

Recommendations:

1- Investigate the molecular and cellular mechanisms by which bromelain influences the gene expression of digestive enzymes, focusing on analyzing protein interaction networks and cellular signaling pathways using advanced techniques such as single-cell RNA sequencing and quantitative proteomic analysis to better understand the relationship between nutrition and epigenetic modifications.

2- Development of bromelain as an adjunct therapeutic protocol for metabolic disorders, with determination of optimal dosing.



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for the Manuscript Entitled:

The effect of the quality of nutrition on gene expression of protease enzyme and activating the immune system in mice balb / c.

This certificate is valid from the date of application in 08/06/2024

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CURRICULUM VITAE

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Date of Birth and Place

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EDUCATION INFORMATION

Academic degrees	Institution	Date Graduation
Bachelor's	Anbar University. Iraqi	2010
Master's	Tikrit University. Iraqi	2019
PhD	Erciyes University. Türkiye	2025

ADMINISTRATION POSITION

Year	**Institution**	**Job Position**
2011	Ministry of Higher Education and Scientific Research – University Of Anbar	Lecturer

FOREIGN LANGUAGE: Arabic and English language

Professional experience :

- Five years of experience presenting medical and nutritional programs on satellite channels.
- Ten years of experience presenting news bulletins and reading documentary reports.
- Presenter of the "Ain Al-Ilm" program, which specializes in broadcasting the latest educational news in Iraq.
- News anchor on the official Iraqiya channel.
- Broadcaster on Samarra Satellite Channel.
- News director on Baghdad Satellite Channel.

- Owner of a well-known nutrition clinic in Iraq, Mr. Diet Nutrition and Fitness Clinic, in Ramadi. Provides nutritional advice, prescribes diet plans, and performs liposuction and body sculpting procedures.

Certificates:

- Bachelor of Food Science and Biotechnology - University of Anbar - College of Agriculture (top student in the college)
- Master of Food Science and Biotechnology - Human Nutrition - Tikrit University - College of Agriculture
- Certificate in International Scientific Publication in Reputable Journals - issued by the IFAD platform - University of Portsmouth - UK
- Certificate of Participation in the Seminar on Publishing in Highly Reputable Journals at Clarivate - issued by the Scientific Supervision and Evaluation Authority - Ministry of Higher Education
- First Practical Conference for Graduation Research of Food Science Students in Iraqi Universities
- Certificate in Recent Developments in Food Manufacturing - issued by the University of Baghdad - College of Agriculture
- Certificate in the Seminar on the Use of Gums and Gels in Food Manufacturing and Other Fields - issued by the University of Baghdad - College of Agriculture
- Certificate in the Workshop (Analyzing Family Trees in Counseling for Human Genetic Diseases) - issued by the University of Fallujah - College of Science
- Certificate in the Workshop (Local Manufacturing of Electrical Spinning Systems and the Most Important Parameters of Nanofiber and Its Use in Producing White Light) issued by the College of Science Applied Sciences - Hit
- Certificate in the conference (The Impact of the Corona Pandemic on Local and Global Animal Production) - College of Agriculture - Tikrit University
- Certificate in the Chemical Safety and Security Course issued by Anbar University - Continuing Education Center 2019
- Certificate in the symposium (COVID-19 Pandemic: What's New?) - issued by the College of Medicine - Anbar University - Continuing Education Unit - 2020

- Certificate in the workshop (DNA and RNA Sequencing Testing) - issued by the College of Applied Sciences - Hit 2020
- Certificate in the workshop (Causes of Environmental Pollution in Haditha District and Methods of Mitigating Its Impact) - issued by the College of Basic Education - Haditha 2020
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