FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

THE INVESTIGATION OF EFFECTS OF HERBAL EXOSOMES ON HEPATIC LIPID METABOLISM IN OBESE RATS

Diyar Ali MAHMOOD

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DEPARTMENT OF BIOLOGY

Molecular Biology

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Author
Diyar Ali MAHMOOD

Supervisor
Asst.Prof. Dr. Zeynep TUZCU

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THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

		Signature
Supervisor:	Asst.Prof. Dr. Zeynep TUZCU Firat University, Faculty of Science	Approved
Chair:	Prof.Dr. ÖkkeşYILMAZ Firat University, Faculty of Science	Approved
Member:	Assoc. Prof. Dr. Mehmet GÜVENÇ Adiyaman University, Faculty of Arts and Science	Approved

This thesis was approved by the Administrative Board of the Graduate School on

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Signature

Assoc. Prof. Dr. Kürşat Esat ALYAMAÇ
Director of the Graduate School

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I hereby declare that I wrote this Master's Thesis titled “The Investigation of Effects of Herbal Exosomes on Hepatic Lipid Metabolism in Obese Rats ” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

01 July 2021

Diyar Ali MAHMOOD

PREFACE

Obesity and type II diabetes are a growing global health problem. The increase of health-related problems is unprecedented in both developed and developing countries. Chronic consumed high-fat foods are related to obesity and metabolic syndrome. Feeding a high-fat diet in experimental animals is directly related to metabolic changes such as hypertriglyceridemia, hyperinsulinemia, and glucose intolerance. In this study, obesity formation will be monitored on rats fed with a high-fat diet to investigate the effect of vegetative exosome on obesity. In this review, we will focus on the growing evidence that supports the role of liver pathophysiology and the potential roles as targets of new biomarkers for these cases. I want to express my gratitude to my supervisor Asst. Prof. Dr. Zeynep TUZCU for providing me the opportunity to study this thesis. Her guidance and her help throughout the process have made this thesis conceivable. I want to express my sincere appreciation for Prof. Dr. Kazim ŞAHİN, Prof. Dr. Nurhan ŞAHİN, Assoc. Prof. Dr. Cemal ORHAN, and Assoc. Prof. Dr. Mehmet TUZCU for their collaboration in this study.

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Diyar Ali MAHMOOD

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ABSTRACT

The Investigation of Effects of Herbal Exosomes on Hepatic Lipid Metabolism in Obese Rats

Diyar Ali MAHMOOD

Master's Thesis

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The increase in obesity is unprecedented worldwide. Obesity is associated with many chronic diseases resulting from an imbalance between a strong metabolic syndrome and energy intake and physical activity. Hypertension and obesity are considered significant risk factors in the development of cardiovascular morbidity and mortality. Extracellular Vesicles are potential biomarkers and therapeutic agents due to their function as an intercellular mediator for cellular communication within the liver and other organs. Many studies examining medicinal plants report that they are beneficial in treating diabetes, obesity, and other chronic diseases. This study aims to update data on potential anti-obesity herbaceous plants and review scientific data, including experimental methodologies that include active ingredients and mechanisms to work against obesity in humans. The present invention relates to a product containing a garlic exosome that used to treat diseases. In this study, obesity formation was observed in rats fed a high-fat diet and the effect of garlic exosome on obesity was also investigated. This work focuses on the data that supports the role of extracellular vesicles in liver pathophysiology and potential roles as targets of new biomarkers for these cases. In the study carried out in the experimental research center, 42 Wistar albino rats were used. After adaptation to laboratory conditions, rats were divided into 6 groups with 7 animals in each group. Groups: Control; Control + Exosome I (400 µg/kg); Control + Exosome II (800 µg/kg); High Fat Diet (HFD); High Fat Diet (HFD) + Exosome I (400 µg/kg); High fat diet (HFD) + exosome II (800 µg/kg) in form. The diet in the groups was prepared to be isocaloric. It was calculated to consist of approximately 12% of the energy in the control group and 42% in the HFD groups. According to the results, body weight increased after using the high-fat diet plus Exosome I&II. Glucose, bilirubin, total protein, albumin, globulin, A/G ratio, liver enzymes, TBIL, MDA levels increased significantly ($P<0.05$) after a high-fat diet. Levels of AP2, LPL, NFκB, Nrf2, PPAR-α, SREBP-1c, and TNF-α significantly increased ($P < 0.05$) after using high-fat diet and Exosome I&II. After using a high-fat diet, large accumulation of lipid droplets and degeneration were observed in hepatocytes. Histological study showed typical structure of liver tissues after using Exosome I&II. In conclusion, this study shows the positive effects of garlic exosome in obesity model in rats fed a high-fat diet.

Keywords: Exosome, Extracellular vesicles, Obesity, High-fat diet

ÖZET

Obez Sıçanlarda Bitkisel Eksozomların Hepatik Lipid Metabolizması Üzerine Etkilerinin Araştırılması

Diyar Ali MAHMOOD

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Obezite dünya çapında endişe verici bir oranda artış göstermektedir. Obezite, güçlü bir metabolik sendrom olarak enerji alımı ve fiziksel aktivite arasındaki dengesizlikten kaynaklanan birçok kronik hastalıkla ilişkilidir. Hipertansiyon ve obezite, kardiyovasküler morbidite ve mortalitenin gelişiminde önemli risk faktörleri olarak kabul edilir. Ekstraselüler veziküller, karaciğer ve diğer organlar içinde hücresel iletişim için hücreler arası bir aracı olarak işlevlerinden dolayı potansiyel biyobelirteçler ve terapötik ajanlardır. Yapılan birçok çalışmada Tıbbi bitkilerin diyabet, obezite ve diğer kronik hastalıkların tedavisinde faydalı olduğu rapor edilmiştir. Bu çalışma, obeziteye karşı potansiyel etkili bitkiler hakkındaki verileri güncellemeyi ve insanlarda obeziteye karşı çalışmak için aktif bileşenler ve mekanizmalar içeren deneysel metodolojiler dahil olmak üzere bilimsel verileri gözden geçirmeyi amaçlamaktadır. Mevcut çalışma, hastalıkların tedavisinde kullanılabilen sarımsak eksozomu ile ilgilidir. Bu çalışmada, yüksek yağlı diyetle beslenen sıçanlarda obezite oluşumu izlendi ve ayrıca sarımsak ekzozomunun obezite üzerindeki etkisi araştırıldı. Bu çalışma, Ekstraselüler veziküllerin karaciğer patofizyolojisindeki rolünü destekleyen verilere ve bu vakalar için yeni biyobelirteçlerin hedefleri olarak potansiyel rollerine odaklanmaktadır. Deneysel araştırma merkezinde yürütülen çalışmada 42 adet Wistar albino rat kullanıldı. Laboratuar koşullarına adaptasyondan sonra sıçanlar her grupta 7 hayvan olacak şekilde 6 gruba ayrıldı. Gruplar: Kontrol; Kontrol + Eksozom I (400 µg/kg); Kontrol + Eksozom II (800 µg/kg); Yüksek Yağlı Diyet (HFD); Yüksek Yağlı Diyet (HFD) + Eksozom I (400 µg/kg); Yüksek Yağlı Diyet (HFD) + Eksozom II (800 µg/kg) şeklindedir. Gruplardaki diyet izokalorik olacak şekilde hazırlandı. Kontrol grubunda enerjinin yaklaşık %12 si, HFD gruplarında ise % 42 yağdan oluşacak şekilde hesaplandı. Sonuçlara göre, yüksek yağlı diyet ve Exosome I&II kullandıktan sonra vücut ağırlığı arttı. Glikoz, bilirubin, toplam protein, albümin, globulin, A/G oranı, karaciğer enzimleri, TBIL, MDA seviyeleri, yüksek yağlı bir diyet uygulandıktan sonra önemli ($P<0.05$) artış gösterdi. AP2 , LPL , NFκB, Nrf2, PPAR-α, SREBP-1c ve TNF-α seviyeleri, yüksek yağlı diyet ve Exosome I&II kullanıldıktan sonra önemli ölçüde arttı ($P < 0.05$). Yüksek yağlı diyet kullanıldıktan sonra, hepatositlerde büyük miktarda lipid damlacıkları birikimi ve dejenerasyon gözlendi. Histolojik çalışma, Exosome I&II'yi kullandıktan sonra karaciğer dokularının tipik yapısını gösterdi. Sonuç olarak bu çalışma, yüksek yağlı diyetle beslenen ratlarda sarımsak eksozomunun, obezite modelinde olumlu etkilerini göstermektedir.

Anahtar Kelimeler: Ekzozom, Hücre dışı veziküller, Obezite, Yüksek yağlı diyet

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SYMBOLS AND ABBREVIATIONS

Symbols

µl	: Microliter
g	: Gram
mg	: Miligram
mmol	: Milimole
ml	: Mililiter
dl	: Deciliter
mM	: milimolar
kg	: Kilogram
w/v	: Weight/volume
nmol	: Nano mole
°C	: Celsius

Abbreviations

i.p.	: Intraperitonial
b.w.	: Body weight
HFD	: High fat diet
GLU	: Glucose
BUN	: Blood Urea Nitrogen
TP	: Total protein
ALB	: Albumin
GLOB	: Globulin
A/G	: Albumin/Globulin
ALT	: Alanine aminotransferase
AST	: Aspartate Aminotransferase
ALP	: Alkaline Phosphatase
TBIL	: Total bilirubin
MDA	: Malondialdehyde
EVs	: Extracellular vesicles
MvBs	: Multivesicular bodies
HO-1	: Heme Oxygenase-1
NF-κB	: Nuclear Factor-κB
PPAR-α	: Peroxisome receptor alpha
SREBP1	: Sterol regulatory binding protein
LPL	: Lipoprotein lipase
TNF-α	: Tumour Necrosis Factor alpha
AP2	:AdaptorProtein2

1. INTRODUCTION

Obesity is one of the most common and widespread health and aesthetic problems among different age groups of both sexes in all parts of the world. It is caused by several factors, including those related to some other health problems and diseases. These factors include daily life habits and practices such as eating foods saturated with fat and lack of exercise and water. It could also be due to a disturbance in hormones. Because this problem negatively affects health and people's confidence in themselves, it is necessary to examine their causes and find appropriate solutions, so doctors recommend eating certain foods. It is one of the widespread health problems for populations and of all age groups globally. Obesity significantly increases morbidity linked with coronary heart disease, type 2 diabetes, metabolic syndrome, stroke, and cancers. The presentation and treatment of this problem are essential for health systems to reduce obesity. Doctors and other healthcare professionals have considered both lifestyle and drug therapy interventions to treat obesity. The liver is significant in metabolizing fats. According to the species, it is somehow the fatty acid synthesis center and circulation of fat through fat protein synthesis. Ultimately, the accumulating fat droplets in the hepatocytes make the degeneration of hepatic fatty caused by multiple dysfunctions, including the changes in oxidation, the secreting low lipoprotein of -density, and pathways used to construct fatty acids. Also, the increased pool of non-estrogenic fatty acids can be a significant determinant of pathogenic fatty liver disease. Many pathways of fat metabolism are at least partial, interrelated, and "trans-regulated." The current discussion will focus on triglycerol and fatty acids. The formers are the most widespread form of stored and circulated energy, and triglycerides are the most common non-toxic fatty acids. Both could emerge from four grouping inputs: cytoplasmic triacylglycerol *de novo* lipogenesis, triglycerides from fatty acid protein that are eaten directly from the liver and non-esterified fatty acids excreted by NEFA. The relative significance of the four inputs is based on the differences between the types (for instance, in ruminants, some *de novo* lipogenesis happens compared to adipose tissue. In birds, the reverse is true, in which the liver is the *de novo* lipogenesis main region [1].

Dietary fat is a vital constituent of the human diet. However, the overconsumption of or an imbalance of the fat type could harm health and well-being. Herbal supplements and treatments based on a diet for weight loss are some of the alternatives, and complementary medicines of these medicinal plants and natural products are raw extracts and plant-isolated compounds. Obesity is the primary health problem for all populations and age groups. It results in an inherent rise in mortality and morbidity related to coronary heart problems, diabetes type 2, metabolic syndrome, cancers, and stroke [2,3]. Its prevention and treatment are vital for health systems for obesity reduction and related problems universally. Physicians and other health care workers

regard lifestyle and pharmacotherapy interventions to treat obesity modalities [1]. Garlic (*Allium sativum* L.) is commonly used as a food ingredient and has been used for centuries as a folk medicine because of its noticeable impacts on disease prevention and treatment [2]. It turns out that many of the organic sulfur compounds present in garlic possess many biological activities. Multiple uses of garlic as it treats many diseases and disorders that a person may have including among these benefits: the treatment of colds impotence and reduce alleviated blood pressure and protection from atherosclerosis and the formation of clots in addition to reducing the ratio of cholesterol to blood. There is strong evidence that garlic does not only prevent cancerous tumors; it slows down its growth and uses garlic as an antifungal and bacterial agent, and reduces arthritis, weight loss, with several biological advantages, including hypolipidemic and hypocholesterolemic effects. The advantages also include antioxidant potential and antimicrobial activity. The pungent garlic fractions are, in most cases, moieties with sulfur. However, its two chemicals, such as flavonoids and alken -based cysteine sulfoxides, also impact health [3].

Garlic (*Allium sativum* L.) is known for its commonly used foodstuff and, for many centuries, as a medicine because of its recognized properties of preventing and treating ailments. In China, 33.3 million square meters are used to cultivate garlic, producing more than 500 million tons, which is ½ of the total Asia garlic cultivation area and 1/3 worldwide. Many compounds of organosulfur are found in garlic oil have numerous biological activities. The compounds are the main biological agents: diallyl disulfide (DADS), diallyl sulfide (DAS), allyl methyl trisulfide, and diallyl trisulfide (DATS). Exosomes were first described in the 1980s, which are nanoparticles linked to the membrane released from the inner cell pathways. Initially, exosomes were regarded as a mechanism to eliminate the membrane proteins. They gained recognition as the primary carrier of cellular information. Because its biologically active load modifies the activity of specific target cells, it resulted in a variety of stimulating or inhibitory functional outputs by exosomes such as cell proliferation, programmed cell death cytokine creation immune modulation, and metastasis [4]. As a result, they are another method to contact paraquin and endocrine system to traditional approaches for straight contact with cellular and soluble and receptor hormones receptors for cytokine receptors. The role of exosomes is used differently in the physiology of humans and balance and in making major human illnesses. Moreover, they provide a hopeful source of vital signs linked to diseases and may finally be utilized in the cell-free delivery vectors to targeting biological therapies. Some research works on the role of EVs in the metabolism in humans and its related disease in early stages [5]. However, their ongoing investigation could provide significant visions and likely treatment strategies [6].

In multicellular organisms, cells communicate through extracellular molecules, such as nucleotides, fats, short peptides, or proteins. These molecules are produced outside the cell and bind to the receptors on other cells. They thereby stimulate intracellular signals and modify the

intracellular physiological state of the recipient cells. Exosomes appear in multicellular bodies MvBs produced into the extracellular environment when fused with the plasma membrane. They transfer the contents from donor cells to the recipient, as in figure 1. This produces functional shifts or differences between the target cells exosomes to protect mRNAs regulatory microRNAs. Furthermore, lipid bilayer membrane-coated fats and proteins EVs cellular constructions are produced by different cells, for example, immune, cancerous, and stem cells in physiological materials such as malignant ascites EVs and blood urine saliva milk amniotic fluid are a significant part in the balance formation, immune response cancer development tissue, and vascular vascularity because it works as a primary mediator for cellular communication [7].

2. LITERATURE REVIEW

The extracellular vesicles are vesicles that are membranous released from prokaryotic and eukaryotic cells in to extracellular space. Based on its cellular origin, it can be classified into a number of subcategories. The diameter of the exosomes is 30-100 nanometers and is produced in the endosome system and released into extracellular space. This happens when the late endosomes merge with the plasma membrane first discussed during the retina maturation [8].

The lower vesicles exhibit another EVs type. They arise from the plasma membrane. This includes ev microscopes mvs of heterogeneous shape, of 50 -1000 nm and objects or programmed cell death typically bigger and show 1 to 4 microns [9].

It is produced from dying cells during program cell death, and many different cell types produce EVs such as hematogenic cells, b lymphocytes, and stem cells dcs [10].

Furthermore, EVs were recognized and extracted from several body fluids: urine, blood plasma, serum saliva in addition to spinal cord media [11]. This entails their release *in vivo*. Although primarily thought that the elasticity of damaged tissues was mainly reliant on stem cells by synchronizing the signal between the local immune system and epithelial at the injury place, recent results show that the experiential capacity to regenerate the stem cells is because of producing their nanoparticles new actors in tissue repair processes [12].

The release of the extracellular vesicle was first known as retinal cells, which matured in sheep in 1983 for the presence of miRNA in these vesicles. The exosomes may be converted into proteins in the target cells. This conversion entails that exosomes change genetic information. This discovery, alongside the concomitant development of miRNAs research, showed a recent enormous rise in the number of sheets on exosomes. History of exosomes secret sacs recognized as exosomes were first exposed almost before 30 years. However, given just slightly more than cellular trash cans annihilate exosomes of unwanted molecular components. There are few studies on them in the last ten years. Nonetheless, over the past few years, as evidence begins to gather that vesicles resemble signal loads that contain specific groups of cells from protein fats and genetic material shifted to other cells in which their work and function could alter. In multicellular organisms, cells connect by extracellular molecules such as nucleotide fats, proteins, or short peptides. These molecules are sent outside the cells by cells and bound to the other cell receptors, thus stimulating intracellular signals and modifying the physiology of the recipient cells. These eukaryotic cells also launch into their complex extracellular structures called membrane vesicles, encompassing numerous proteins, fats, and nucleic acids. It can affect cells that meet these constructions in more multifaceted ways. Although it has been recognized for several decades, it is generally named microscopic particles. In semen, it is called the prostate. The membrane vesicles have long been believed to be cell debris. The phenomenon of exosomes

and their broader participation in the intercellular signal emerged from this image in the past decade [13].

Exosomes are a specific subclass of the sacral membrane vesicles. They are formed in endosome compartments, multicellular endosomes, with internal vesicles that fill and store molecules in the structures attached to the membranes. Endosomes are generally a middle chamber linking the plasma membrane in which the internal vaccination of extracellular molecules and the compartments of lysosomal particles happen. However, 25 years ago, groups from Philip Stahl in the USA rose Johnstone in Canada using very sophisticated tests for the pulse. The electron airport is defined in matrix retinal cells in red blood cells (RBC) late multicellular endosomes collapsing with plasma membrane rather than lysosomes. Its contents include many tiny sacs outside the cell. In 1987, exosomes were anticipated to classify these vesicles in internal bodies. The first significant progress in subsequent years was associated with large-scale protein examination techniques, allowing us to reveal that exosomes are a specific cell compartment rather than random cell debris [14].

Protein analysis exosome synthesis was first achieved on exosomes that are then veiled by the dendritic cells. Exosomes from different types of cells are grouped into the exocarta compendium. These works revealed that exosomes have no random groups of intracellular proteins. However, a specific set of rare proteins comes from the plasma pathway, endocytic membrane, and cytosol with minimal proteins from other parts. For example, these results proved that living cells produced exosomes produced and proved their internal origin. Exosomes began to clean the in vitro not only tissue cultures from immune cells but also epithelial cells. Cancer cells begin to turn out that the exosomes of a cell captured by another cell and change the information to the last cell. These notes deliver a source for the idea that exosomes are a new type of intercellular message. In addition, the presented at least in the laboratory use considerable amounts of concentrated exosomes [15].

2.1. Secreted Vesicles and Intercellular Communications

2.1.1. Extracellular Vesicles

Extracellular vesicles (EVs) are vesicles that are membrane-produced from prokaryotic and eukaryotic cells. They are secreted into the extracellular environment. Based on their subcellular origin, they could be grouped into many subtypes. "Exosomes are 30-100 nm in diameter generated within the endosomal system and secreted into the extracellular space upon fusion of late endosomes with the plasma membrane first described during reticulocyte maturation [16]. Shedding vesicles are a different class of EVs plasma membrane-originated. This ev population involves microvesicles (mvs) whose heterogeneous shape and size are about 50 1000 nm while

the apoptotic bodies or blebs are commonly bigger, whose size range is1 4 µm. The latter is created by dying cells during processes of apoptotic. Many cell classes produce EVs such as hematogenic cells, such as b lymphocytes and stem cells.

2.1.2. Biogenesis and Extracellular Release of Exosomes

Exosomes are made in the endocytic track incorporating extracellular or cell charge retention of new endosomes and extra debasement arranging or reusing way, while growing from early to late endosomes. Vesicles are framed in the luminal lives by internal blockage of the inward film, as in Figure 2.1. Luminal endosomes are then inserted into intraluminal vesicles (ILVs), a particular element that gives them the name of multivesicular bodies (MvBs). In the ilv development measure, proteins, fats, and nucleic acids are arranged into vesicles which depend on their lipids MvBs intertwine with the lysosome to destroy their payload or converge with the plasma layer goes into ilvs into extracellular space. Then, the delivered ILVs are changed over to exosomes, while the intraluminal vesicles (ILVs) are created by the early endosome layers and additional maturing into the endosal pit.

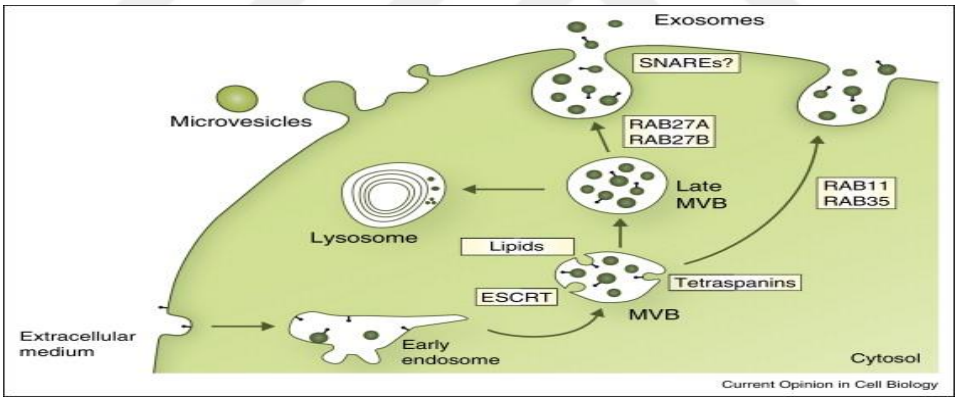

Figure 2.1. Exosome biogenesis (Endosomal inside parts loaded up with ILVs named multicellular bodies (MVB) and numerous atoms, for example, ESCRT machine proteins tetraspanins and ceramide fats may take an interest in ILVs biogenesis Depending on their destiny, MVB cells can either combine with the lysosome for intracellular corruption or with the plasma layer to discharge their ILVs as exosomes in the extracellular space. Several rat proteins appear to be associated with the exchange of MvBs to the plasma layer. Interestingly, mvs bud straightforwardly from the plasma film endorsed and changed photograph.

2.1.3. Biophysical Properties of Exosomes

Exosomes are recognized by their morphological profile size thickness and atomic structure and The Exosomes are recognized by their morphological profile size, thickness, and atomic structure, and these attributes are generally used to recognize them from different EVs. Exosomes' morphology and size have been broadly concentrated by oppressing refined exosome

arrangements to electron microscopy (em) showing up as vesicles 40 120 nm covered by a lipid bilayer. It was later distinguished as an efficiently instigated antique because of the breakdown of the vesicle film. When utilizing cryo-em, exosomes usually appear round. Since all vesicles contain fat, exosomes can glide in sucrose slopes at a precise thickness of 1.13 g ml to 1.19 g ml, while larger vesicles are the point at which the apoptotic bodies coast at a higher thickness going from 1.24 1.28 g ml [8].

2.1.4. Nucleic Acids in Exosomes

Protein and fat exosomes are in (mRNA) messenger and little decoded RNA as Valadi et al. 2007 stated [11]. These scholars and others possibly indicate that the mRNA exosomal and miniature RNA (miRNA) molecules can be moved to have cells where they can change levels of protein transcription. The cutting edge succession NGS of exosomal RNA uncovered to the existence of mRNA and minor ncRNA types, for example, miRNA piwi intelligence RNA (piRNA) little atomic RNA (snoRNA) and little nuclear RNA (snRNA) just as the move of RNAs (tRNA) inside exosome arrangements. Exosomes are possibly advanced in particular sorts of RNA compared to cell RNA, and their RNA arrangement might not be the same as cell proposing precise focusing of RNA screening anywhere into exosomes. Exosomes from various cell types could change from the same charge from miRNAs; a typical component for focusing on miRNAs to exosomes can likewise be found [17].

2.1.5. Physiological Function of Exosomes

From the start, exosomes were viewed as jars of cell litter to dispose of pointless or out of date plasma layers or the cytoplasmic proteins. It was discovered that the transferrin receptors were moved from the plasma layer to the extracellular space by the applicable exosome mvb system during the development of the retinal cells to the red platelets [18]. It was first proposed that the part of exosomes signals in correspondence starting with one cell then onto the next when it was indicated that lymphocytes b emitted mhc ii exococ expected to initiate cd4 lymphocytes and accordingly intervened the resistant reaction, the exosomes could evoke the immune reaction by actuating the resistant cells including providing the immediate co-peptide of T cells or DCs or moving the antigen to non-industrial nations [19, 20]. Inhibitory impacts on the immune reaction to exosomes incorporate restraint of immune system microorganism actuation hindrance T cells are the common poison of the executioner cell and upgrade administrative lymphocyte movement Thery et al. 2009. Plus, exosomes were examined to take an interest in morphology due to their relationship with affirming their capacity in sign transmission. Moreover, multiple studies have

zeroed in on expected capacities in exosomes focal sensory system cns. It has been seen that numerous cell types, for example, neurons hypothyroid and microglia discharge exosomes that can be moved to the objective cells covered up exosomes of oligodendrocytes, are engaged with managing myelin development because of the inhibitory impact as portrayed exosomes from glia were advanced in synapsin I, and appeared to advance nitrite development in hippocampal neurons and the neuronal endurance of cortical neurons under cell stress conditions [21]. In addition to studying the potential role of exosomes, the function of neural synaptic where the release of exosomes from the somatic-dendritic part of the cortical neurons is regulated by the flow of calcium and glutamic synaptic activity. Besides protein transport, exosomes were shown to transfer mRNA molecules ingested by target cells and translate them into corresponding proteins or small RNA types that operate on the target mRNA and thus interfere and modify gene expression. Horizontal transmission of genetic information via exosomes has been reported as a new intercellular communication mechanism in multiple cell models such as embryonic stem cells, mast cells, glioblastoma cells, endothelial cells, or primary cortical neurons [22]. The investigation of the likely job of exosomes is found in the capacity of neural synaptic; the arrival of exosomes from the physical dendritic piece of the cortical neurons is controlled by the progression of calcium and glutamic synaptic movement. Other than protein transport, exosomes appeared to move mRNA atoms ingested by target cells and interpret them into comparing proteins or miRNA types that work on the objective mRNA and meddle and alter these lines along with these lines quality articulation [23]. Flat transmission of hereditary data through exosomes have been accounted for as another intercellular correspondence system in different cell models, for example, early-stage undifferentiated organisms pole cells and glioblastoma cells endothelial cells or essential cortical neurons.

2.1.6. Exosomes in Pathological Processes

The part in neurodegenerative sicknesses notwithstanding its natural capacity, it is expected that exosomes are participatory in numerous obsessive cycles and pathologies, including malignant growth infection contamination and neurodegenerative illnesses, for example, Alzheimer's sickness promotion and Parkinson's illness, prion sickness (PD) and amyotrophic horizontal sclerosis al despite the distinctions in clinical indications and sub-atomic pathology most neurodegenerative infections share typical highlights, for example, total dead tissue localized necrosis and the gathering of proteins for illnesses that lead to intracellular or extracellular stores in the cerebrum. These incorporate with numerous others β-amyloid and tau in promotion α-synuclein in PD and prion proteins prp in prion and superoxide pyroxide grass infections in als. Surprisingly, these proteins were delivered from cells as a team with exosomes in the laboratory second regular element of these problems is that protein-related infections

spread all through the mind over the long haul after anatomical associations. Among a few elective techniques for work, exosomes have been accepted to add to the spread of pathogenic kinds of protein by moving them between various cells. The part of EVs in bacterial biofilm development and upkeep has additionally been illustrated. Cells of plant extra discharge EVs with transport RNAs safeguard mixes and flagging lipids recommending that plant EVs may work as key middle people in plant-organism connections.

2.1.7. Biogenesis Discharge of Exosomes and Other Extracellular Intercellular Interactions

Vesicles contents and functions of extracellular vesicles isolated from plants extracellular vesicles EVs are small lipid compartments that operate in the long-range transport of proteins and nucleic acids other metabolites. In mammals, EVs are essential means of intercellular communication and play a critical role in modifying immune responses [24, 25]. Plant cells also secrete EVs, especially in response to infection, but the contents of these follicles have not been analyzed, and their functions are unknown. in order to better understand ev plants and their role in defense and signaling, I first took ground-breaking methods to isolate and purify EVs from washing between the leaves of the arabidopsis. Second, I studied the protein and RNA contents of the purified EVs. Protein analysis revealed that arabidopsis EVs are enriched for defense and pressure-related proteins. Consistent with this result, ev secretion was strengthened in response to biotechnology. Moreover, collaboration with the Blake Meyer laboratory at the Donald Danforth center for plant science identified several types of small RNA in antiseptic Ev samples and a surprising enrichment of small RNAs tRNAs of length from 10 to 17 nt. Finally, by studying the interactions between arabidopsis EVs and colletotrichum higginsianum plant pathogenic fungi, I discovered that plant EVs bind to fungal structures and influence the evolution of the natural formation. With these steps, my research represents a breakthrough in plant Ev research [26]. It provides strong evidence of plant involvement EVs in the immune response and indicates that it can trade proteins and RNA in invasive pathogens.

2.1.8. What are Extracellular Vesicles EVs

Extracellular vesicles are tiny structures encompassed by layers radiating from a cell in the general climate. In each of the three everyday issues, EVs are an effective method for intercellular correspondence. They go about as defensive compartments for significant distance transport of sign particles, including proteins, nucleic acids, fats, and different metabolites. For the most part, EVs are assembled by how they are shaped and partitioned into one of three classes: apotheosis tiny bodies or exosomes. The missional bodies are the biggest and the most homogenous of the three classes.

Microfilms mvs sprout from the plasma film straightforwardly, though exosomes are made inside endosome compartments known as multicellular bodies MvBs and discharged from the cell when MvBs converge with the plasma layer [27-29]. EVs in mammals have attracted much attention in recent years due to their ability to modify immune responses and functional inter-cell RNA molecules. The biological properties of its EVs inspired scientists to use them for both therapeutic and diagnostic uses.

Protein release assumes a vital part in every single eukaryotic cell, and it is grouped into two subgroups: regular protein discharge cps and whimsical protein emission ups in plant cells; cps comprises of a few stages: 1. solvent proteins with an n-terminal found sign peptide SP and layer proteins are incorporated in the endoplasmic reticulum 2. recently combined proteins are moved to the Golgi contraption for additional adjustment 3. the proteins are then conveyed to the Golgi [30] network tgn were arranged into the plasma film pm for discharge and 4. the combination of secretory vesicles with pm prompts the arrival of proteins into pmor to extracellular space. Ups then again is answerable for emitting proteins that do not convey any sp or other cell materials into extracellular space, which may prompt the development of extracellular vesicles EVs in mammalian cells. EVs assume a significant job in intervening intercellular correspondence by conveying biomolecules, including protein charges, and advancing RNA to the objective cells through the extracellular space [31, 32]. To date, three kinds of EVs have been recognized in well-evolved creatures, including one exosome got from a combination of multivesicular body mvb combination to the uprooting of tiny, infinitesimal particles beginning straightforwardly from the pm and three that produced adipose bodies during cell demise. Not at all like mammalian cells, plant cells have a cell divider which may forestall the arrangement and capacity of EVs [33, 34].

Furthermore, developing proof recommends that plants also produce EVs that partake in different capacities, including safeguarding against microorganisms. Utilizing progressed cell protein and atomic procedures, late examinations have improved our insight into EVs in plants. In this survey, we initially sum up late information about the nature and emission of various kinds of EVs in plants, just as feature the fundamental controllers associated with these cycles. We, at that point, proceed onward to talk about the novel capacity of plant EVs in plant guard and beneficial interaction.

Despite the global nature of EVs, most research on these structures is limited to animal cells, which may sound strange given that the initial observations of EV secretion come from fungi and plants. Shortly after, the tem vesicle-like structures were trapped between the plasma membrane and the cell wall in the fungal strands that appeared in the transmission electron microscope. These structures were called lomasomes or internal bodies. Two camps quickly formed ideas about the origins of lomasomes [35]. Some researchers have suggested that

developments in plasma caused the implantation of parts of the membrane and the formation of vesicles. In contrast, others believed that lomasomes were formed when MvBs merged with the plasma membrane. Others still admit the heterogeneity of luminal tumors and consider that both forms of biogenesis can produce similar structures. Unable to determine the precise mechanism facing the possibility of both processes occurring, the term prominent bodies pmbs was invented. Very similar to the term EVs, pmbs can be used widely to refer to any membrane structure between the plasma membrane and cell wall. Moreover, pmbs are divided into two categories: lomasomes which were derived from endosomal membranes, and plasmalemmasomes which were directly derived from the plasma membrane. The proliferation of pmbs in fungal strands of researchers led to the assumption that these structures were unique to fungi and had a role in cell wall formation. This idea was quickly challenged as other scientists began observing pmbs in algal and plant cells, pmbs were observed in collaboration with wheat mesophyll cells tracheal elements from beets and squash cucurbita maxima series bean plant cultured carrot cells and willow slippery. These studies could not agree on whether plant pmbs were primarily plasmalemmasomes or lomasomes. However, some provided clear images of what appeared to be MvBs fused with the plasma membrane. Regardless of how these plant pmbs are produced, their purpose remains a mystery. Most scientists believe that plant pmb cells similar to fungi helped grow and expand by building materials and enzymes to the cell wall. However, some plant pmb components were discovered when the cell wall synthesis did not exist, suggesting that they either lasted for a long time after delivering their payload or possessed another function.

Furthermore, vesicles emitted from MvBs appeared outside the cell wall in intercellular voids [36, 37]. Plant EVs have been incorporated into papillae for more than fifty years now. Scientists have explored the purpose of plant EVs using TEM all this time; it became clear that EVs play a role in plant immunity. Remarkably, this idea dates back to one of the first reports on plant pmbs, claiming that plumia in healthy wheat cells was associated with resistance to stem rust. Tested this claim by infecting varieties of sensitive and rust-resistant wheat fungi puccinia graminis tritici or puccinia recondita and examining cells for the presence of lomasomes. This study found lomasomes in affected cells of both resistant and exposed varieties but noted that these structures were missing in the absence of the pathogen. Lomasomes accumulated in cells penetrated by fungi and localized in areas containing fungal structures. Upon close examination, lomasomes appeared to contain small vesicles. The authors suggested that these structures were a direct response of the plant cell to the pathogens and contributed to changes in the cell wall. The first in a long series of temporary studies that observed PMBS accumulation in affected cells.

2.1.9. The Nature of EVs in Plants

For the most part, EVs are characterized as lipid vesicles with a lipid bilayer containing cell vesicles delivered by the cell into the extracellular space. In plants, EVs principally go about as defensive chambers for the vehicle of numerous materials among cells and add to plant development and improvement protective reaction just as the concurrence of the plant microorganism. At present, EVs are thought to speak to a class of heterogeneous vesicles that have various beginnings and play exceptional capacities. Potential wellsprings of EV arrangement in plants incorporate exhibition exocyst-positive organelle MvBs vacuoles and autophagosomes. EVs were first seen in quite a while during the 1960s. The transmission electron magnifying lens tem synthetically fixed island considers shown variable volumes of EVs and combination of MvBs with pm. from EVs diffusive detachment techniques were created and executed [26, 38]. For the most part, low radial powers are applied to eliminate cell flotsam, and jetsam and afterward, fast powers are utilized to gather EVs dependent on thickness. For instance, in 2013, joe and his associates separated grape-like nanoparticles gelns from grape juice utilizing differential centrifugation techniques and sucrose inclination strategies. Countless film vesicles encompassed by a nanolayer measurement 50-300 nm were available in a piece of the geln as seen under cryo-em. Later a few examinations likewise detailed the presence of EVs in the mode of dust grains in olives extracellular liquids for sunflower seedlings and coconut milk. In past exploration, the expected tainting of broken cells is the fundamental impediment in the exactness of deciding EV charge by protein examination. As of late, a more complex technique has been created to confine EVs from apoplastic washing of arabidopsis tissues utilizing vacuum channel centrifugation and centrifugation at an overly quick speed. With this technique, moderately unadulterated EVs were disengaged with little contamination [39, 40]. In this manner, protein examination demonstrated that these detached EVs contained lipids metabolites proteins and little RNA ribosomal RNA associated with the guarded reaction. Furthermore, ev discharge was improved when plants were tainted with pseudomonas by infusion or treated with salicylic corrosive.

2.1.10. The Important of Exosome

Exosomes act as protective carriers; exosomes contain vital molecules and can be secreted through different types of cells in body fluids, such as plasma, urine, semen, and breast milk. Since exosomes consist of a lipid bilayer, they represent an effective protective barrier for molecules within the ureter. For example, enzymes in plasma or other body fluids; RNA or protective proteins may quickly degrade, leading to inconsistent results. Exosomes, with the help of the vesicle structure, protect their contents from exposure to external environments.

Consequently, previous studies have shown correlations between exosomes of biological contents and various cardiovascular diseases, a prerequisite for biomarkers [41]. Exosome-based biomarkers are a diagnostic tool in cardiovascular diseases. The contents and quantities of exosomes are variable under different pathological cardiovascular conditions. Therefore exosomes may act as new diagnostic biomarkers. Exosomes included miRNAs and proteins, are the most widely investigated components. In addition, other charges such as fats can also act as possible biomarkers. Exosomes based miRNA extracellular circulating miRNAs can be detected in body fluids, including blood. Moreover, exosomes and other EVs are the primary sources for the rotation of miRNAs. Most miRNA plasma is concentrated in exosomes and bound to RNA binding proteins compared to freely circulating miRNA. Therefore, miRNAs with exosomes carry great potential as a new diagnostic biological sign for heart disease. Exosomes are defensive transporter exosomes containing fundamental particles and can be emitted through various kinds of cells in body liquids, such as plasma pee semen and bosom milk. Since exosomes comprise a lipid bilayer, they speak to a possible defensive hindrance for particles inside the ureter. For instance, in light of catalysts in plasma or other body liquids, RNA or defensive proteins may effectively debase, prompting flimsy outcomes. Exosomes, with the assistance of the vesicle structure, shield their substance from openness to external conditions. Thus past investigations have indicated connections between exosomes of natural substance and different cardiovascular infections, which is essential for biomarkers. Exosome-based biomarkers as a symptomatic instrument in cardiovascular illnesses the substance and amounts of exosomes are variable under various obsessive cardiovascular conditions. Accordingly, exosomes may go about as new analytic biomarkers. Exosomes included miRNAs and proteins, are the most broadly researched parts. Moreover, different charges, for example, fats, can likewise go about as potential biomarkers. Exosomes based miRNA extracellular flowing miRNAs can be recognized in body liquids, including blood. Also, exosomes and different EVs are the real hotspots for the turn of miRNAs. Most miRNA plasma is packed in exosomes and bound to RNA restricting proteins. In this way, miRNAs with exosomes convey incredible potential as another symptomatic natural sign for coronary illness.

2.1.11. Exosomes Functions in Plant

It has been demonstrated that both eukaryotic and prokaryotic cells release the EVs in the extracellular environment initially the function of the EV secretion was thought to be the disposal of unwanted compounds from cell [2] it is now known that instead of being waste carriers EVs also act as signal compounds that exchange components between the cells. A large proportion of EV studies have focused on mammalian systems, and their function has been well summed up, in mammals plays a variety of roles that include the immune system of the nervous system,

reproductive cancer, fetal growth, repair of bone calcification and liver balance. Other studies have been reported to function even in other organisms. For example, EVs shown to influence the development of mating behavior in elegant balanitis. The role of EVs information and maintenance of biological bacteria has also been clarified. The 2006 Beveridge plant cells also release EVs that contain RNAs defense compounds and the signal-to-fat ratio, indicating that plant EVs may act as a critical mediator in the microbial interactions, regarding the function of plant EVs concerning plant defense as well as symbiosis.

Both eukaryotic and prokaryotic cells discharge the EVs in the extracellular environment at first, the capacity of the Ev emission was believed to be the removal of undesirable mixes from the cell [34]. It is presently realized that as opposed to being waste transporters, EVs additionally seen as sign mixes. An enormous extent of EVs is considered zeroed in on mammalian frameworks, and their capacity has been very much summarized in vertebrates assumes an assortment of jobs that incorporate the invulnerable arrangement of the sensory system [42], conceptive malignancy, fetal development, fix of bone calcification and liver equilibrium. Different investigations have been accounted for to work even in different living beings. For instance, EVs appeared to impact the improvement of mating conduct in rich balanitis [43, 44]. EVs in developing and upkeep organic microorganisms and has likewise been explained, and the plant cells additionally discharge EVs with RNAs guard mixes. Also, the sign-to-fat proportion demonstrating that plant EVs are a vital go-between in microbial cooperation, concerning the capacity of plant EVs comparable to plant protection just as beneficial interaction.

2.1.12. The liver

- In a Figure 2.2, the liver is situated in the upper right of the stomach pit, beneath the diaphragm, over the stomach, right kidney, and intestine. The cone-formed liver is a dim rosy earthy colored organ that weighs around 3 pounds. There are two particular wellsprings of blood supply to the liver, including the accompanying:
- The oxygenated blood streams from the hepatic course
- Nutrient-rich bloodstreams from the hepatic entry vein

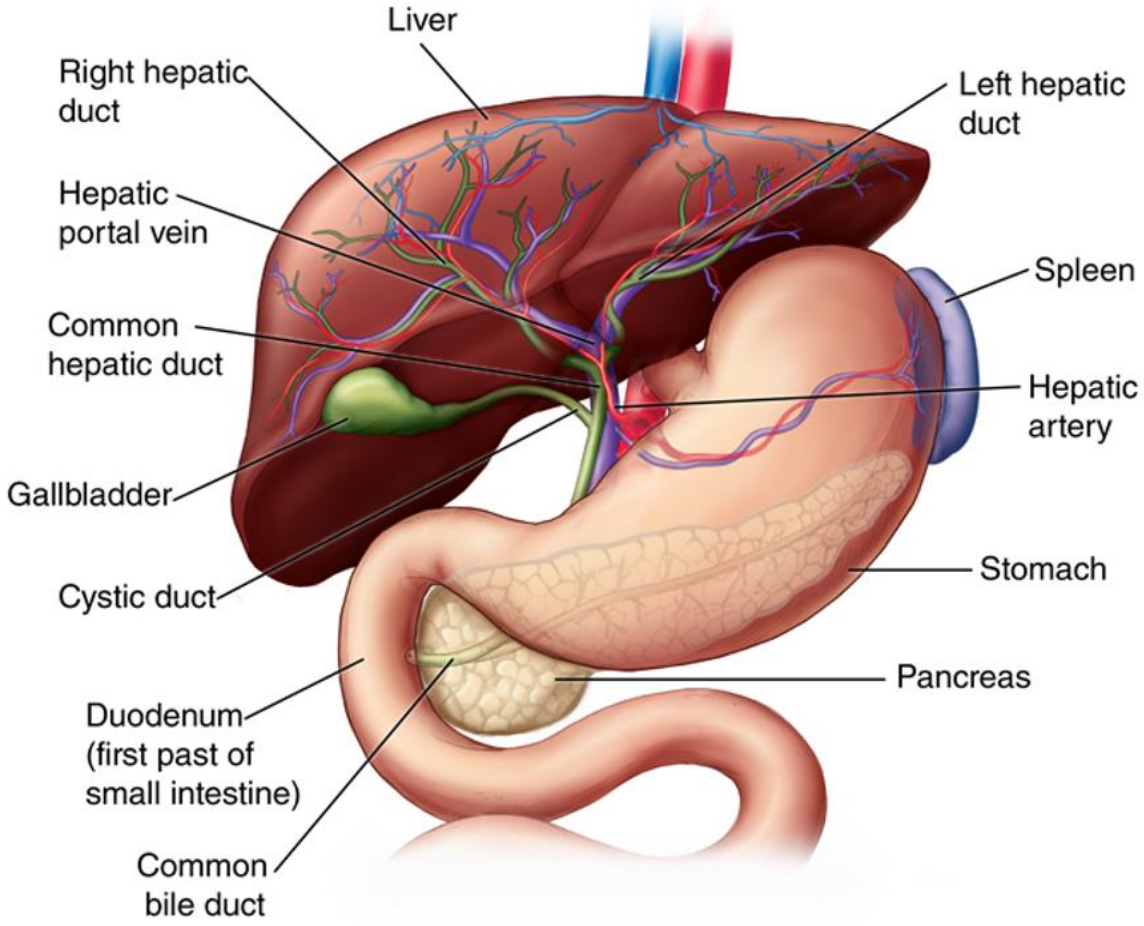

Figure 2.2. Picture showing the parts of the liver (The liver has two large sections, called the right and the left lobes. The gallbladder sits under the liver, along with parts of the pancreas and intestines.

The liver holds about a large portion of a liter (13%) of the body's blood supply out of the blue. The liver comprises two principal projections. Both are made out of 8 sections comprising of 1000 lobules (tiny projections). These lobules are associated with little conduits (tubes) that interface with more prominent channels to frame the hepatic pipe. The hepatic channel transports bile made by liver cells to the gallbladder and duodenum (the initial segment of the small digestive tract) through the bile conduit.

2.1.13. Effects on histological change of the liver;

Dietary fat is an essential component of the human diet, but too much fat or an imbalance in the type of fat can have adverse effects on health and wellbeing.

A. Chronic consumption of excessive calories in addition to physical inactivity promotes obesity, diabetes, and metabolic syndrome.

B. Obesity is associated with excessive secretions of fats in tissues such as the heart, hepatocytes, the liver, and fats leading to toxicosis and inflammation.

C. The accumulation of fat in the liver that is the digestive liver, leads to non-alcoholic fatty liver disease and a growing health problem in the obese population and more severe liver diseases such as non-alcoholic fatty hepatitis, cirrhosis, and cancer.

D. Although excessive consumption of saturated fat sfa enhances the storage of fats and inflammations, polyunsaturated fatty acids (pufas) play a protective role by controlling the synthesis and oxidation of sfa and monounsaturated fatty acids (mufas) and reducing the hepatic fat content.

Features of fats associated with the risk of cardiovascular disease. The objective of this article is to highlight recent studies that implicate fatty acid synthesis as a goal to control the severity of risk factors associated with diabetes and metabolic syndrome mets. Fat is an essential part of the human eating routine, yet an excessive amount of fat or an awkwardness in the sort of fat can effectively affect wellbeing and prosperity:

a. Chronic utilization of over the top calories notwithstanding actual inertia advances stoutness diabetes and metabolic disorder.

b. Obesity is related to unnecessary discharges of fats in tissues, for example, the heart hepatocytes, the liver, and fats prompting toxicosis and aggravation.

c. The gathering of fat in the liver that is the stomach related liver prompts non-alcoholic greasy liver illness and a developing medical issue in the hefty populace and more genuine liver infections, for example, non-alcoholic greasy hepatitis cirrhosis and disease.

d. Although unnecessary utilization of soaked fat sfa improves the capacity of fats and aggravations polyunsaturated unsaturated fats pufas assume a defensive part by controlling the combination and oxidation of sfa and monounsaturated unsaturated fats mufa and decreasing the hepatic fat substance .

e. Features of fats related to the danger of cardiovascular illness. This article aims to feature late examinations that ensnare unsaturated fat combination as an objective to control the seriousness of danger factors related to diabetes niddm and metabolic condition mets.

2.1.14. EVs in Human Metabolic Disease

EVs are involved in a growing group of human diseases, including conditions related to obesity and metabolic syndrome. Because they are ubiquitous and stable in much human biological fluid because their contents reflect the properties of parent cell EVs, especially exosomes, their messenger miRNA has been explored as an accessible source of new diagnostic and diagnostic biomarkers in metabolism [45]. Cytometry analyses revealed a quantitative increase in prevalent EVs in obesity and associated disease conditions, including insulin resistance, diabetes, and non-alcoholic fatty liver disease (nafld) biogenesis, and extracellular release of exosomes. Exosomes are generated along the endocytic pathway, including

extracellular or cellular charge uptake to early endosomes and additional degradation sorting or the recycling pathway. During ripening from early endosomes to late endosomes, intraluminal vesicles (ilvs) are formed by internal congestion of the endosomal membrane lumina of these endosomes is then filled with ilvs a distinctive feature that with the name of the multivesicular bodies MvBs during the ilv formation process proteins fats and the nucleic acids are sorted into vesicles depending on their lipids MvBs fuse with a lysosome to degrade their payload or merge with the plasma membrane to release ilvs to extracellular space released ilvs are referred to exosomes. The biogenesis and release of the exosomes from eukaryotic cells. Intraluminal vesicles ilvs are formed by the inner membrane of the early endosomes and the mutation in the inner cavity. Indonesian interior parts filled with ilv are called multicellular bodies' mvb, and many molecules such as escort machine proteins tetrabanine and ceramide fats may participate in ilv biogenesis. Depending on their fate, MvBs can either fuse with lysosomes for intracellular degradation or with the plasma membrane to secrete their ilvs as exosomes in extracellular space. Many rap proteins appear to be involved in the transfer of MvBs to the plasma membrane. In contrast, mvs bud directly from the plasma membrane. Approved and modified photo.

EVs are associated with developing human illnesses, including a gathering of conditions identified with heftiness and metabolic disorders. Since they are omnipresent and stable in numerous human natural liquids because their substance mirrors parent cell EVs' properties, particularly exosomes, their courier miRNA has been investigated as an open wellspring of new symptomatic and indicative biomarkers in digestion [46, 47]. Cytometry examines uncovered a quantitative expansion in standard EVs in weight and related illness conditions, including insulin opposition diabetes and non-genital greasy liver infection nafld biogenesis and extracellular arrival of exosomes. Exosomes are created along the endocytic pathway that incorporates extracellular or cell charge take-up to early endosomes and extra corruption arranging or reusing pathway. During aging from early endosomes to late endosomes, intraluminal vesicles ilvs are shaped by internal blockage of the endosomal film lumina of these endosomes is then loaded up with ilvs a particular element called multivesicular bodies MvBs during the ilv development measure proteins fats, and the nucleic acids are arranged into vesicles based on the lipids MvBs in them combined with a lysosome to debase their payload. Alternatively, they converge with the plasma layer to deliver ilvs to the extracellular space. Delivered ilvs allude to exosomes. The biogenesis and arrival of the exosomes from eukaryotic cells. Intraluminal vesicles ilvs are framed by the internal layer of the early endosomes and the transformation in the inward pit. Indonesian internal parts are loaded up with ilv called multicellular bodies mvb and numerous atoms; for example, escrt machine proteins tetrabanine and ceramide fats may be in ilv biogenesis. Many rap proteins have all the earmarks associated with the exchange of MvBs to the

plasma layer. Interestingly mvs bud straightforwardly from plasma layer affirmed and altered photograph.

2.1.15. Heme Oxygenase-1 (HO-1)

Heme oxygenase (HO) is a rate-restricting compound in the catabolism of heme. It is of three kinds of (HO) isoforms (HO-1, HO-2, HO-3). Heme oxygenase-1 (HO-1) is a 32-kDa protein encoded by the Hmox1 quality, is a pressure responsive catalyst engaged with insurance from infection and rebuilding of homeostasis. HO-1 is regularly communicated at low levels in many tissues, yet it is exceptionally inducible by various improvements. HO-1 corrupts free heme to yield equimolar measures of three items: the gas carbon monoxide (CO) [48], iron, which prompts the statement of heavy chain (H-) ferritin (an iron-sequestering protein), and biliverdin, which is changed over to bilirubin by biliverdin reductase (BVR). The fundamental biologic capacity of HO-1 is to keep away from the aggregation of exceptionally harmful free heme and likely add to its broad impacts in insurance against a vast range of illnesses and in reestablishing homeostasis. First, HO-1 articulation is initiated by an incredible number of agonistic pressure improvements. This likely identifies with the enormous number of DNA-restricting groupings that exist in the 5′ untranslated administrative district of Hmox1, to which record elements can tie to manage its pace of record Second HO-1 capacity intercedes an expansive scope of helpful impacts by debasing heme, notwithstanding by creating three distinctive organically dynamic particles [CO, iron (Fe), and biliverdin] assume an indispensable job in guarding and fixing oxidative pressure-actuated harm. The final results of heme breakdown are generally powerfully cell reinforcement and calming. Third, a considerable scope of practical activities is interceded by the distinctive 'arms' of the HO-1 framework and recommend that HO-1 has a focal part in insurance and the restoration of homeostasis after a broad scope of pathologic conditions [49].

2.2. Nuclear Factor-κB (NF-κB)

NF-κB is a gathering of basically related and developmentally moderated proteins that have a place with the Rel family and are directed through carrying from the cytoplasm to the core in light of cell incitement. NF-κB is communicated in the cytoplasm of basically all cell types, where its movement is constrained by a group of administrative proteins, called inhibitors of NF-κB (IκB). IκBα, IκBβ, IκBγ, and Bcl-3. NF-κB is associated with cell reactions to upgrades, for example, stress, cytokines, free extremists, bright light, and bacterial or viral antigens. NF-κB proteins can be isolated into two classes dependent on arrangements C-terminal to the RH space Members of the five star comprise of NF-κB1 and NF-κB2 that incorporated as huge forerunners, called (the NF-κB proteins p105, p100) have long C-terminal area likewise these natural NF-κB1/p105 and NF-κB2/p100 proteins contain ankyrin rehash (which act to restrain these particles). At that point, it preparses to create NF-κB subunits, p50 from p105 and p52 from p100, by the demonstration of proteolysis individually. The individuals from this top-notch are not activators of record for the most part, except when they structure dimers with individuals from the below-average of Rel/NF-kB. The inferior incorporate incorporates c-Rel, RelA (p65), and RelB [11]. Besides, the p50 and p52 homodimers likewise tie to the atomic protein Bcl-3, framing potent transcriptional activators [50]. Ongoing perceptions demonstrated that both IκBα and IκBγ transport between the core and cytoplasm inside NF-κB-IκB buildings, these edifices are equipped for dislodging NF-κB from target DNA locales and moving it back to the cytoplasm [47]. NF-κB actuation is firmly directed by signals that debase (IκB) inhibiter of NF-Kb.

2.3. Nuclear Factor Erythroid 2-related Factor 2 (Nrf2)

Atomic factor erythroid-2-related factor 2 is a record factor, an individual from the cap 'n' collar/essential leucine zipper (CNC-bZIP) protein family [51]. An immense range of improvements actuate Nrf2, and it is controlling various cell measures. The guideline of the Nrf2 movement is perplexing and multifactorial. Inside the cells, the guideline of Nrf2 levels can be controlled at the transcriptional and post-transcriptional levels [52]. Keap1 communicates with Nrf2 to frame a complex at the present circumstance; Keap1 goes about as an inhibitor of Nrf2. Plus, the Keap1 Nrf2 inhibitor can likewise associate with Cul3-E3-ligase [49], which intervenes in Nrf2 corruption in the ubiquitin-proteasome framework [53]. During oxidative pressure, Nrf2 is initiated through altering the three significant cysteine deposits of Keap1, and conformational changes happen in the Nrf2/Keap1 mind-boggling and prompting progression of Nrf2, consequently Nrf2 movement to the core inside the core, Nrf2 ties to ARE with the assistance of sMaf protein [50]. In a straightforward succession way, The Nrf2-sMaf complex ties to the cancer

prevention agent reaction component (ARE 5′-TGACXXXGC-3′) in the advertiser area of Nrf2 target qualities [54]. Succeeding investigations delayed the characteristic of proteins encoded by the ARE quality succession incorporating qualities engaged with cancer prevention agent reactions, drug detoxification, NADPH recovery, and digestion guidelines [55]. Nrf2 also controls the declaration of the ROS and xenobiotic detoxification, proteins associated with NADPH recovery, heme digestion, glutathione (GSH), and thioredoxin (TXN) cancer prevention agent framework, in this manner assuming a focal job in keeping up the redox homeostasis of the cell.

2.4. Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α)

Peroxisomes are subcellular organelles found in most plant and animal cells that perform diverse metabolic functions. PPARs are transcription factors that belong to the Superfamily of nuclear hormone receptors [56]. Other family members include thyroid, vitamin D, glucocorticoid receptors, retinoic acid, estrogen, and several other proteins involved in xenobiotic metabolism. PPARs act on DNA response elements as heterodimers with the retinoid X receptor (RXR). PPARs' natural activating ligands include lipid-derived substrates. The family of PPARs is represented of the following three subtypes: PPARα, PPARβ/δ, and PPAγ they are essential in the energy metabolism but, differ in the spectrum of their activity [57]. Transcription factor Peroxisome proliferator-activated receptor alpha/PPAR-α is mainly participating in enhancing and activating mitochondria and peroxisome fatty acid β-oxidation pathway, transport of fatty acid, and production of hepatic glucose. It is involved in metabolic control and inflammatory reactions in the kidney, liver, and other tissue. Furthermore, it is essential in the lipid and lipoprotein synthesis, increasing the PPAR-α expression is identified in those tissue with high oxidation of fatty-acid including the liver, kidneys, heart, and some other tissues and cells such as muscle cells, adipose cells, and immune cells as well as PPAR-α activated by fibrates and polyunsaturated fatty acid [58].

2.5. Protein SREBP1

In the previous twenty years, non-alcoholic Fatty liver infection has spread heightened to disturbing levels. This condition influences 30% of everybody, up to 75% of patients with type 2 diabetes. Hepatic Fat gathering is related to impeded insulin signals. Hepatic glucose creation is not repressed. In any case, insulin keeps up the arrangement of new fat in a known cycle. As specific protection from insulin, decreasing liver hindrance, Glucose creation, and expanded fat arrangement lead to a mix of Hyperglycemia and hyperlipidemia. The SREBP-1c6 record factor manages the new lipogenesis measure in the liver in light of expanded insulin. The essential

record factors are leucine annular IUD. The Sahab family is made as an antecedent and restricting the layer of the endoplasmic reticulum. Within sight of Proper signs, SREBPs moving to Golgi, where it is broken, develops structure, which is moved to the core and enacts the objective quality articulation. SREBP-1a and SREBP-1c are equivalent types of similar quality. Regulates glycolic and fat catalysts Such as L-pyruvate kinase (Pklr), unsaturated fats (Fasn), steroil-CoA desaturase 1 (Scd1), glycerol 3-phosphate mitochondria acyltransferase 1 (Gpam), and caretylylase acetyl-CoA (ACAC). SREBPs, are record factors that go about as the essential controller of qualities that control the greasy homeostasis of fat cells to meet the prerequisites of a living being. SREBPs have appeared to assume critical parts in fat digestion with nourishment, cell development, energy pressure, ammation, and so forth from physiological and neurotic cycles. In vertebrates, SREBP1 and SREBP2 proteins travel from the endothelial organization to the Golgi mechanical assembly because of cholesterol hardship. It is handled by the insightful division of the protein and coordinated to the core, where it animates the declaration of qualities included fundamentally in sterols and the biosynthesis of unsaturated fats. The liver's strange aggregation of fatty oils brought about by expanded *de novo* lipogenesis and liquor actuated greasy liver infection and insulin obstruction. The administrative components restricting protein (SREBP1), a significant transcriptional controller of lipid arrangement, will undoubtedly be inert of the forerunners connected to the endoplasmic reticulum (ER). Because of insulin signals, SREBP1 is moved from ER to Golgi in a COPII-subordinate way, handled by protease in Golgi, and afterward moved to the core to animate lipid quality articulation nonetheless, the systems hidden SREBP1 upgraded action stay large, and insulin protection from diabetes is muddled.

2.6. Lipoprotein lipase (LPL)

Lipoprotein lipase (LPL) is a multifunctional chemical delivered by numerous tissues, including fat tissue, cardiovascular and skeletal muscle, islets, and macrophages. LPL is a compound that diminishes the hydrolysis pace of the fatty oil center (TG) of TG-rich lipoproteins, microns, and extremely low thickness lipoproteins (VLDL). LPL-animated response items, unsaturated fats, and mono-glycerol are incompletely ingested locally by tissues and treated unexpectedly; for instance, they have been put away as unbiased fat in fatty tissue or oxidized or put away in skeletal and heart muscles or as a cholesterol ester and TG in macrophages. LPL is coordinated at a record, post-interpretation, and post-interpretation levels in a tissue-explicit way. All supplements and hormonal levels affect the guideline of LPL, and an assortment of proteins communicating with LPL have been recognized to manage the particular action of tissues. Generally, LPL is a magnificent chemical that unmistakably adds to the digestion of characteristic lipoprotein, the conveyance and utilization of tissue substrates, and numerous different parts of

heftiness and other metabolic issues identified with energy balance, insulin activity, and bodyweight guideline.

2.7. Adaptor protein (AP2)

The AP-2 group of record factors comprises of five unique proteins in people and mice: AP-2α, AP-2β, AP-2γ, AP-2δ, and AP-2ε. The proteins have a chiral loop shape at the carboxylate end, which intercedes a focal locale of focal mistiness and DNA official. The amino end contains the conditional area. All AP-2 mammalian proteins aside from AP-2 are encoded by seven exons and offer a particular area structure. AP-2 record factors are frequently distinguished in the core, as they tie to the objective chain and direct the record of target qualities. It has likewise been demonstrated that AP-2 proteins meddle with other sign transduction pathways. The action of AP-2 proteins can be controlled at various levels: their preparing potential, DNA authority, subcellular restriction, and corruption abilities can be changed. The administrative components incorporate post-translational changes, for example, protein kinase A phosphorylation, de-oxygenation, and oxidation guideline, just as actual communication with various proteins, either that responsive proteins tweak the movement of AP-2 proteins or are influenced by their capacity by authoritative to AP-2 proteins.

2.8. Tumour Necrosis Factor alpha TNF-α

TNF-α encodes a multifunctional incendiary animating cytokine in the tumor corruption factor (TNF) family. Macrophages principally emit this cytokine. It can tie to and demonstrate through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine controls a broad scope of natural cycles, including cell expansion, separation, apoptosis, lipid digestion, and coagulation. This cytokine has been involved in various illnesses, including immune system infections, insulin obstruction, and malignancy. Studies in mice have additionally recommended the neuroprotective capacity of this cytokine. Human TNF-a will be a 17 kDa cytokine made of 157 amino acids with 76 amino presence of the eye. It can be found in either dissolvable or untreated film structure (233 amino acids.- - Protein forerunners have, as of late, been the subject of broad investigation, and the metal protein inhibitors may have remedial potential in illnesses in which TNF-α is known to have a pathophysiological job. TNF-an is available) In the physiologically dynamic structure as an isomorphic organ with a sub-atomic mass of 52 kDa.

3. MATERIAL AND METHOD

This section explains the general strategy of this investigation, such as rats group organization, feeding, and methods of treatments. Furthermore, describe the materials, solvents, and methods performed for HPLC (High-Performance Liquid Chromatography) test and Western blot techniques for analysis of liver tissue samples.

3.1. Study Strategy

In this thesis, 42 Wister-rats, age: 8 weeks, weight 200 ± 20 gram, are set up as rodent lures and held at 22 °C in a managed climate with a light-dull time of 12:12 hours and water not obligatory. All tests performed under the National Institutes of Health Guide for care and utilization of research facility creatures proved by the Ethics Committee of Firat University (meeting date 22/05/2019 and numbered 120). In the following transformation to research center conditions, the rodents are arranged into six gatherings, with each gathering contains seven rodents, as demonstrated (Table 3.1).

Table 3.1. The materials and their amount

Control group:	12% of calories were fed with the standard diet.
Exosome I group:	400 (µg/kg)
Exosome II group:	800 (µg/kg)
HFD group:	42% of calories were fed with the standard diet.
HFD+ Exosome I group:	400 (µg/kg)
HFD+ Exosome II group:	800 (µg/kg)

After twelve weeks of treatment, the bodyweight of all rats was measured; after sacrificing the rats, blood and liver samples were taken and prepared for further laboratory investigation. At the end of the experiment, various biomarkers such as blood tissue metabolic parameters (glucose, lipid profiles, insulin, urea, creatine, CK, ALT, AST, MDA), liver tissue parameters PPARa, SREBP1c, LPL, aP2, TNFα, NF-kB, Nrf-2. Serum parameters will be determined by the Western Blot technique of Blood Analyzer Device (Autoanalyser, Samsung), MDA HPLC device (Shimadzu) in our laboratory. Histopathological examinations will be carried out in Fırat University Faculty of Medicine Pathology Department laboratories. All mathematical information will be introduced as mean ± standard deviation (SD). Single-direction ANOVA will break down

the test values. P <0.05 worth will be considered genuinely critical. All will be performed utilizing Statistical Analysis Software (SPSS 21.0).

3.2. Laboratory Analysis

There is further analysis performed in the following sections.

3.2.1. HPLC

Karatepe's method was used for measuring serum and liver tissue MDA concentrations, with a CTO-10 AS VP column and 30 mM KH2 PO4, a Shimadzu UV-vis SPD-10 AVP detector, and methanol (82.5: 17.5, v/v, pH 3.6) at a flow rate of 1.2 ml/min. Column waste was monitored at 250 nm. Tissue homogenization (10%, w/v) in 10 mM phosphate buffer (pH 7.4) was made and has undergone centrifuging at 13,000 x g for 10 minutes at 4 °C. The resulting supernatant was kept at -80 ° C for the MDA test [80]. Serum and liver tissue MDA concentrations were analyzed by the strategy depicted by the Karatepe technique, with a Shimadzu UV-vis SPD-10 AVP indicator, a CTO-10 AS VP segment, and 30 mM KH2 PO4 and methanol (82.5: 17.5, v/v, pH 3.6) at a stream pace of 1.2 ml/min. Column waste was observed at 250 nm. Tissue homogenization (10%, w/v) in 10 mM phosphate cradle (pH 7.4) was arranged and centrifuged at 13,000 x g for 10 minutes at 4 °C. The subsequent supernatant was gathered and kept at - 80 ° C for MDA assessment [59].

3.2.2. Analysis of Proteins

Samples of liver tissue were homogenized, and total protein amounts of homogenates were calculated. Levels of HO-1, Nrf2, PPAR-α, NF-κB, LPL, AP2, SREBP-1c, and TNF-α protein in which the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blot methods were utilized to be determined. SDS-PAGE and Western blot analyzes of liver tissue samples were done according to the method applied by Sahin's method [81]. Fresh or frozen tissues were homogenized with a glass homogenizer in a 1:10 (w / v) homogenization solution (PBS). Homogenates underwent centrifuging at 15,000 xg for one hr at + 4 ° C in a refrigerated centrifuge. The first supernatants were taken into Eppendorf tubes and stored at –80 ˚C. The pellets were resuspended in homogenization solution [25 mMTris-HCl (pH = 7.4), 2% TritonX –100 and 1% SDS 0.1 mM PMSF,] added in an equal volume. It was incubated for 2 hours at +4˚C, and the homogenates have undergone centrifuging at 15,000 x g period of one hour, 60 in the refrigerated centrifuge at +4 ˚C. Meanwhile, total protein concentrations were measured with a nano-measuring device (MaestroGen, Las Vegas, NV, USA). The second

supernatants obtained were taken into microcentrifuge tubes and kept at -80 ˚C for SDS-PAGE and Western blot analysis [60].

3.2.3. SDS-PAGE Analysis

The appropriate separation gel solution was prepared to form acrylamide gel look Table 3.2. This gel solution, which was thoroughly mixed after the preparation, was poured between two parts of the cassette, compressed from certain parts through an automatic pipette, and approximately 1 cm space was left for the loading gel. The loading gel was prepared and poured into the gap to allow the acrylamide monomers to polymerize, and combs were placed. Since the loading gel polymerized very quickly, care was taken to make the processes in a short time. The comb was removed from the gel, the polymerization of which was completed. During this process, care was taken not to spoil the slots occurring in the gel and from which the samples will be loaded. The cassette made of glass plates was placed in the electrophoresis tank. Protein solvent solution; prepared as 0.125 M Tris (pH 6.8), 0.002 % Bromophenol blue, 2 % SDS, 20 % glycerol and 10 % mercaptoethanol. An equal proportion of solvent was added to each protein sample, taken approximately 150 µl, and mixed. Depending on the width of the comb gap, 10--20 µl were transferred from the mixture we prepared. A sufficient amount of tank solution was added to the tank. Later, glass cassettes placed in the system (Bio-Rad, Tetra cell System, Mini-PROTEAN®, UK) were electrophoresed for electrophoresis. Then, the immunoblotting phase was started.

E. Solutions used in SDS-PAGE
F. 1.5 M Tris-HCI (pH 8.8)
G. 0.5 M Tris-HCI (pH 6.8)
H. 10% Sodium dodecylsulfate solution (SDS)
İ. 30% Acrylamide / Bisacrylamide solution
J. 10% Ammonium persulfate solution (APS)
K. N, N, N ', N', -tetramethyl-ethilendiamine (TEMED)
L. Glycerine
M. 2-ß- Mercaptoethanol
N. 0.05% Bromophenol blue solution

Table 3.2. Preparation of Gels for SDS-PAGE

Separating (separation) Preparation of gel (12%)	**Amount**
Distilled water	3.35 (ml)
(pH 8.8)1.5 M Tris-HCI	2.50 (ml)
10% SDS	100.00 (µl)
Acrylamide / Bis (30%)	4.00 (ml)
Ammonium persulfate (10%)	50.00 (µl)
TEMED	5.00 (µl)
Total	10.00 (ml)
Preparation of stacking gel (4%)	**Amount**
Distilled water	6.10 (ml)
(pH 6.8)0.5 M Tris-HCl	2.50 (ml)
SDS (10%)	100.00 (µl)
Acrylamide-Bis (30%)	1.30 (ml)
Ammonium persulfate (10%)	50.00 (µl)
TEMED	10.00 (µl)
Total	10.00 (ml)

3.2.4. Western Blot Analysis

Polyacrylamide gels were taken to blot the proteins in the gel into the nitrocellulose membrane (Schleicher and Schuell, Inc., USA) for transfer (blotting). The membranes were exposed without gaps, and they were placed in the blotting device wrapped with filter papers and saturated with immunoblot buffer solution [NaH2PO4.2H2O (0.025 M), Na2HPO4.12H2O (0.075 M), NaCl (1.45 M)]. The apparatus placed in tanks filled with cooled buffer solution was treated with an electric current of 175 mA for 90 minutes. In this way, the protein transfer from the gel to the membrane was created. To prevent non-specific reactions after blotting was completed, nitrocellulose membranes in Petri dishes were washed three times for five minutes on the shaker with buffer solution to coat (blocking) the non-protein-bound portions of the nitrocellulose membrane. Nonspecific binding was obstructed with 1% fresh bovine serum albumin (BSA) in 100 mM NaCl, 20 mM NaH2PO4 (pH: 7.2), 20 mM Na2HPO4,) buffer. As antibodies in the reaction process with specific primary antibodies HO-1 antibody NF-κB, antibody Nrf2, antibody PPAR-α, antibody SREBP-1c, antibody LPL, antibody AP2, and antibody TNF-α (Abcam Inc., CA, USA) were used.

Primary antibodies were utilized in the buffer with a 0.05% Tween – 20 in a 1: 1000 ratio. Nitrocellulose membranes. Its incubation was overnight at +4 ˚C with primary antibodies. In the

next step, nitrocellulose membranes were washed five times with buffer solution for five minutes. After the washing process was completed, nitrocellulose membranes were incubated with peroxidase-conjugated secondary goat-anti-rabbit immunoglobulin prepared in a buffer with 0.05% Tween-20 in a 1: 1000 ratio. In the next stage, nitrocellulose membranes were washed five times with buffer solution for 5 minutes. A diaminobenzidine (DAB) solution prepared in the ratio of 0.03-0.05% was used in a 1 M Tris (pH: 7.4) buffer to display the bands. As a result of the reaction with DAB, the bands on the nitrocellulose membranes soon became visible. Following a reaction time of 5-10 minutes, the bands colored with DAB were visible, and the nitrocellulose membranes were thoroughly washed. After the nitrocellulose membranes were thoroughly dried, the relative densities of the tapes were taken for analysis. The relative densities of the bands were tested by the software program [Image Analyzer System (Image J; National Institute of Health, Bethesda, USA)].

4. RESULTS

This section shows and explain the results of western blot analysis for determining protein expression levels of (AP2, HO1, LPL, NFκB, Nrf2, PPAR-α, SREBP-1c, and TNF-α) and also Histological study to see sections of the extracted tissue and compare it to the control group to see accumulation and deposition of fat droplets and steatosis in liver cells. Also, to see the effects of garlic exosome on normal biochemical parameters, HFD, and rats with diabetes. In all the elements' effects, western blots were performed again at any rate for triple times, and a representative blot appeared. β-actin was incorporated as a protein stacking control. Measurable correlations are shown with an alternate superscript which is (a-c) in a similar line ($P < 0.05$; *ANOVA and Turkey Test, which is a post-hoc test).

4.1. Effects of Garlic Exosome on Liver Tissue HO-1 Protein Levels

In this study, the protein expression level of HO-1 significantly decreased ($P < 0.05$) in both HFD group and Exosome I group, relative the control group. However, administration of garlic exosome I to the HFD+Exosome I group not showed significant effect. On the other hand, administration of Garlic exosome II to the HFD+Exosome II substantially improved ($P < 0.05$) protein level of HO-1, as shown in Figure 4.1.

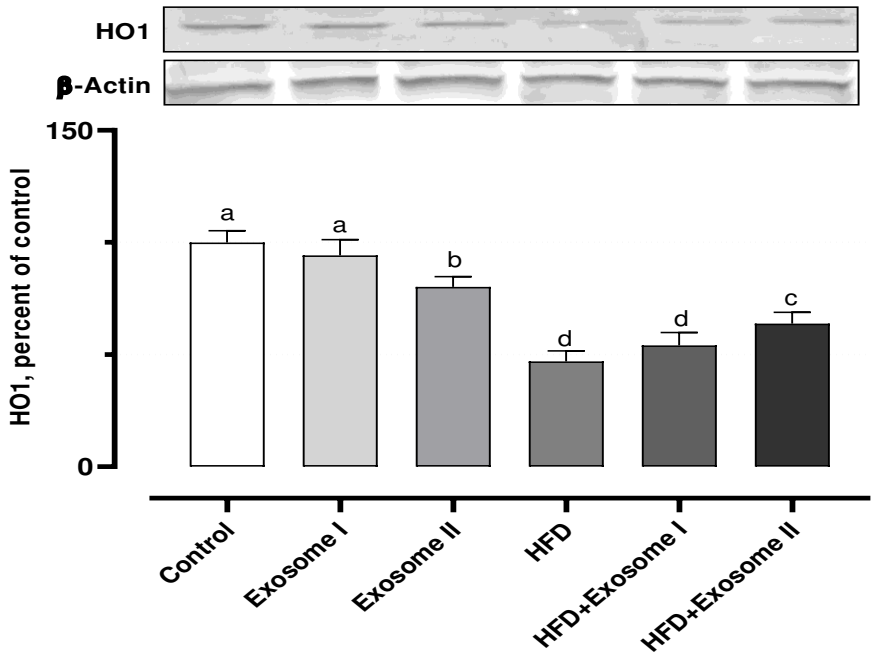

Figure 4.1. Effect of garlic exosome on the HO-1 protein level

This figure shows the effects of garlic exosome administrations on rat liver tissue HO-1 protein levels in rats HFD model.

4.2. Effects of Garlic Exosome on Liver Tissue NF-κB Protein Levels

In the present investigation, there is a significant rise in the protein expression level of NF-κB in the HFD ($P < 0.05$) than in the control group. Whereas in both Exosome I group Exosome. In group II, the NF-κB protein expression did not show significant change compared with control. On the other hand, administration of Garlic exosome to HFD+Exosome I and HFD+Exosome II meaningfully diminished ($P < 0.05$) protein expression level in both groups and down-regulated NF-κB protein levels, as can be seen from Figure 4.2.

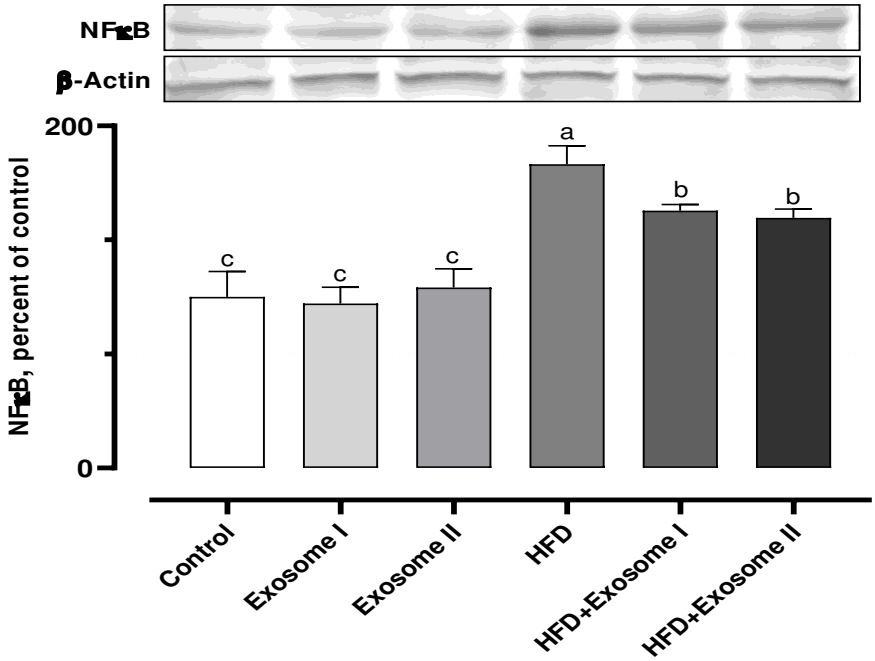

Figure 4.2. Effect of garlic exosome on the NF-κB protein level

This figure shows the effects of garlic exosome administrations on rat liver tissue NF-κB protein levels in rats HFD model.

4.3. Effects of Garlic Exosome on Liver Tissue Nrf2 Protein Levels

In this examination, in the HFD group, the protein expression level of Nrf2 substantially diminished ($P < 0.05$) compared to the control group. While in the Exosome I group Exosome II group, the Nrf2 protein expression did not differ significantly compared to the control group. However, administration of Garlic exosome to HFD+Exosome I and HFD+Exosome II groups notably increased ($P < 0.05$) the Nrf2 protein transcription level in either group and improved the Nrf2 protein expression levels, as shown from Figure 4.3.

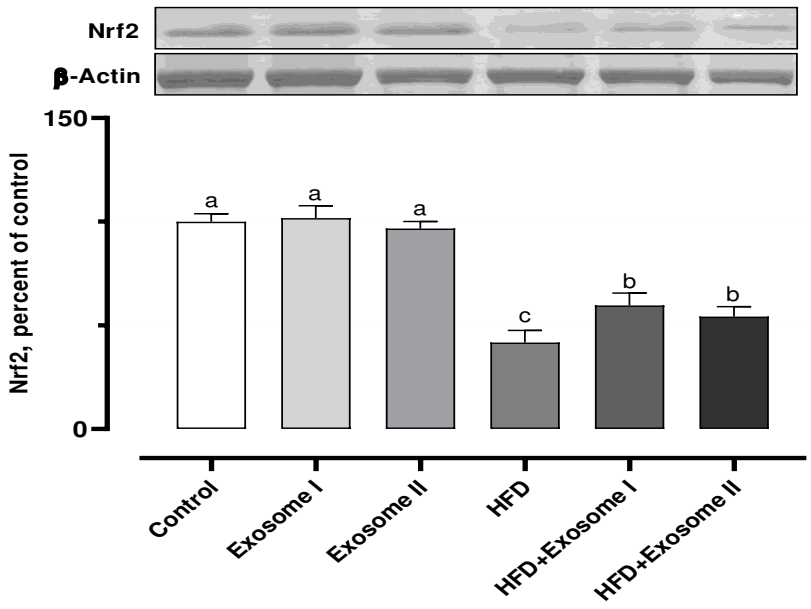

Figure 4.3. Effect of garlic exosome on the Nrf2 protein level

This figure shows the effects of garlic exosome administrations on rat liver tissue Nrf2 protein levels in rats HFD model.

4.4. Effects of Garlic Exosome on Liver Tissue PPAR-α Protein Levels

In this investigation, the PPAR-α protein expression level in the HFD group considerably decreased ($P < 0.05$) about the controls. However, no significant difference appeared in the PPAR-α protein level in the Exosome I group Exosome II group compared with control. Conversely, administration of garlic exosome to HFD+Exosome I and HFD+Exosome II substantially increased ($P < 0.05$) the PPAR-α protein transcription level in both groups and restored the PPAR-α protein levels, as shown below from Figure 4.4. This figure shows the effects of garlic exosome administrations on rat liver tissue PPAR-α protein levels in rats HFD model.

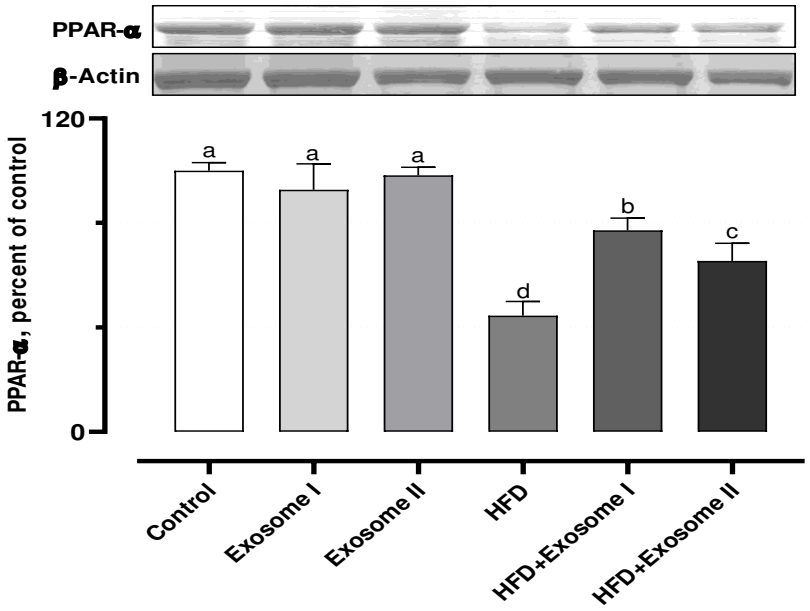

Figure 4.4. Effect of Garlic exosome on the PPAR-α protein level

This figure shows the effects of Garlic Exosome administrations on rat liver tissue PPAR-α protein levels in rats HFD model.

4.5. Effects of Garlic Exosome on Liver Tissue SREBP-1c Protein Levels

In this analysis the protein expression level of SREBP-1c markedly rised ($P < 0.05$) in the HFD group in relation to the controls. However, in Exosome I group Exosome II group the SREBP-1c protein expression not showed significant changed in comparison to the controls. In contrast, Garlic exosome administration to the HFD+Exosome I and HFD+Exosome II dramatically decreased ($P < 0.05$) the SREBP-1c protein expression level in both groups and declined the expression of SREBP-1c protein, as can be seen from Figure 4.5.

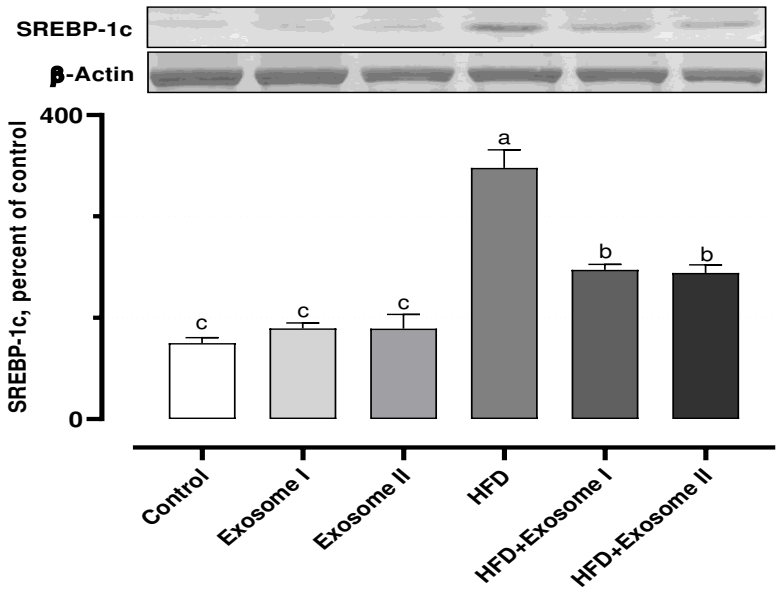

Figure 4.5. Effect of Garlic exosome on the SREBP-1c protein level

This figure shows the effects of Garlic Exosome administrations on SREBP-1c protein levels in rat's liver tissue in the HFD model.

4.6. Effects of Garlic Exosome on Liver tissue LPL Protein Levels

In this study, the protein expression level of LPL noticeably elevated ($P < 0.05$) in the group of HFD than the control group. While, the protein level of LPL displayed not important different in both the Exosome I group Exosome II group compared with the controls. However, administration of Garlic exosome to HFD+Exosome I and HFD+Exosome II noticeably dropped ($P < 0.05$) the LPL protein expression level in both of the groups and reduced the protein levels of LPL, especially in the HFD+Exosome II normalized LPL protein level, as shown below from Figure 4.6.

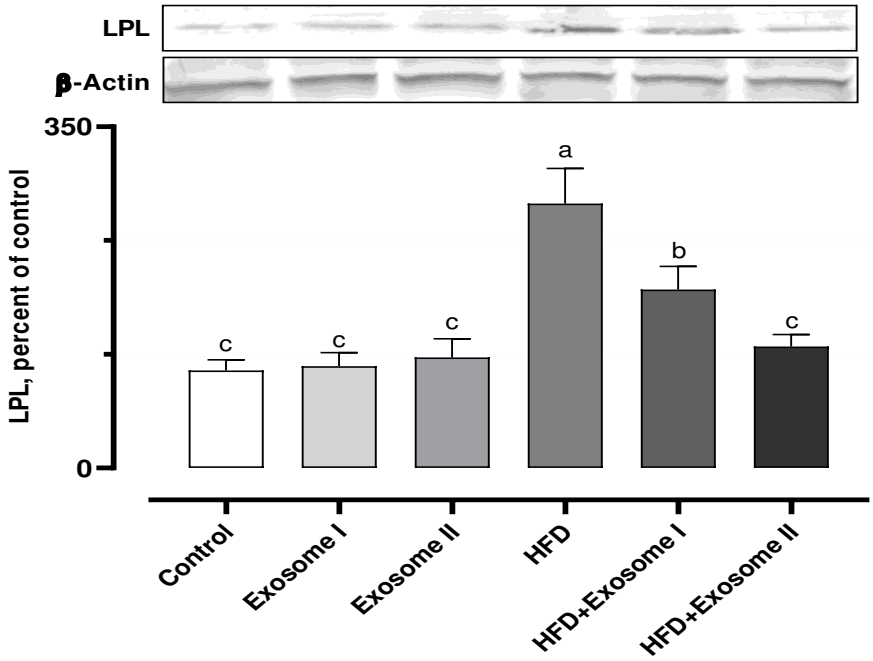

Figure 4.6. Effect of Garlic exosome on the LPL protein level

This figure shows the effects of Garlic Exosome administrations on rat liver tissue LPL protein levels in rats HFD model.

4.7. Effects of Garlic Exosome on Liver Tissue AP2 Protein Levels

In this investigation, in the HFD group, the protein expression level of AP2 noticeably improved ($P < 0.05$) in comparison to the control group. Whereas AP2 protein level in Exosome I group and Exosome II group did not display significant changes compared to the control group. However, garlic exosome administration to the HFD+Exosome I and HFD+Exosome II notably diminished ($P < 0.05$) protein expression level of AP2 in both groups, as shown in Figure 4.7.

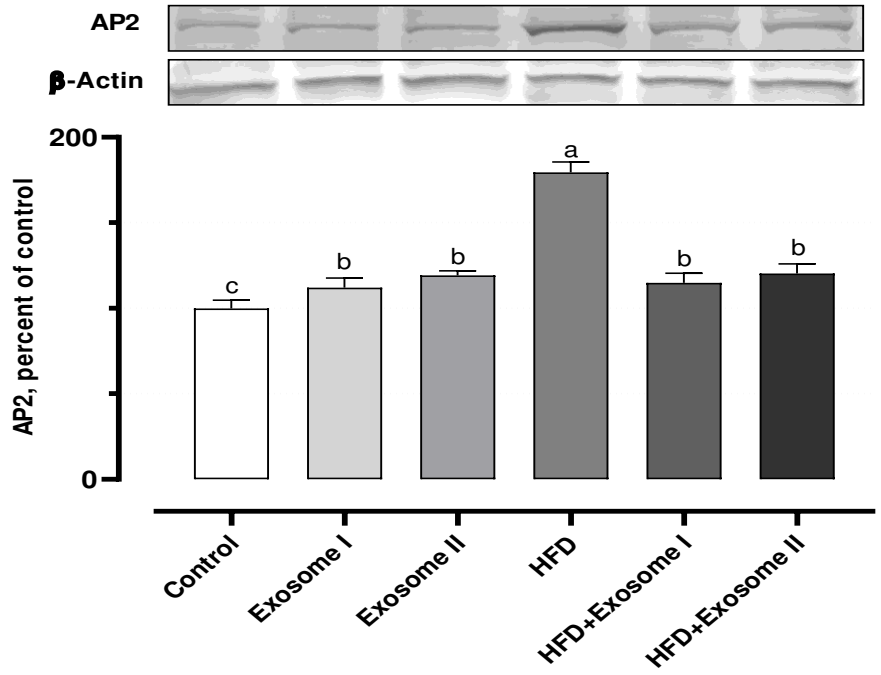

Figure 4.7. Effect of Garlic exosome on the AP2 protein level

This figure shows the effects of Garlic Exosome administrations on rat liver tissue AP2 protein levels in rats HFD model.

4.8. Effects of Garlic Exosome on Liver Tissue TNF-α Protein Levels

In this analysis, the protein expression level of TNF-α significantly rose ($P < 0.05$) in the HFD group more than in the control group. In contrast, in the Exosome I group and Exosome II group, the levels of TNF-α protein expression levels did not show significant changes compared with the control group. However, administration of garlic exosome to both groups HFD+Exosome I and HFD+Exosome II substantially decreased ($P < 0.05$) the protein expression level of TNF-α in both groups, especially in the group of HFD+Exosome II supplementation of Garlic exosome restored the protein level of TNF-α to the normal range, as can be seen from Figure 4.8.

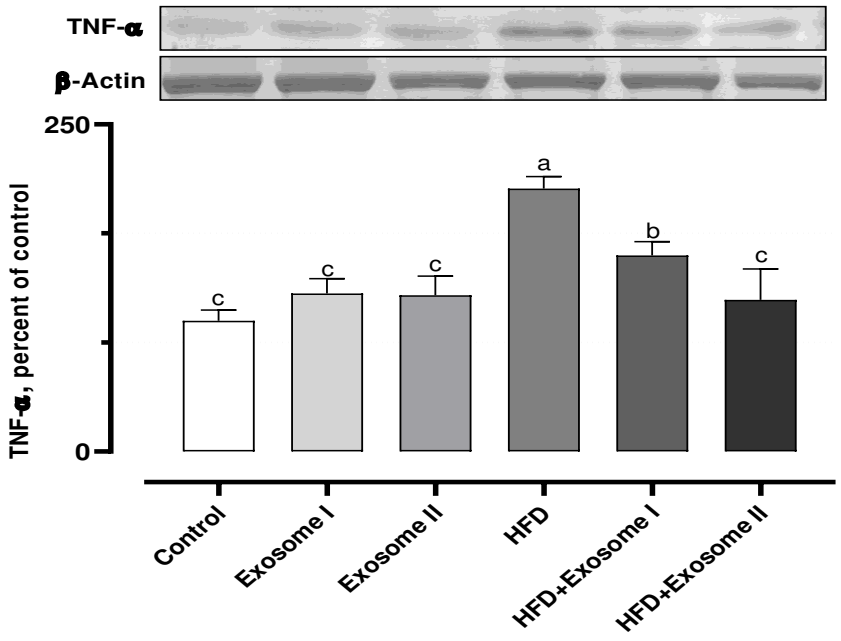

Figure 4.8. Effect of Garlic exosome on the TNF-α protein level

This figure shows the effects of Garlic Exosome administrations on rat liver tissue TNF-α protein levels in rats HFD model.

4.9. Effects of Garlic Exosome on the Rats Body Weight

The Garlic exosome not showed significant effect on rat's body weight at initial bodyweight the measurements, as can be seen in Figure 4.9.

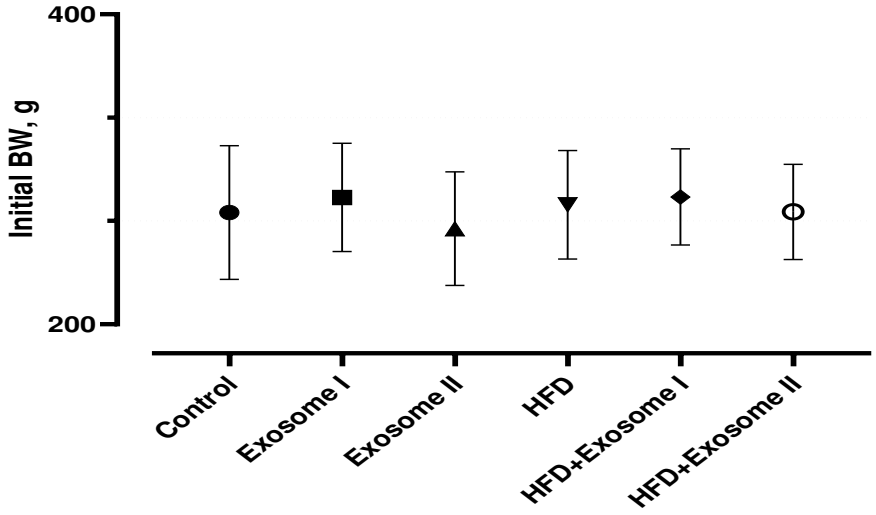

Figure 4.9. Effect of Garlic exosome on initial body weigt

However, the garlic exosome displays a significant effect ($P < 0.05$) on the rat's final body weight, as shown from Figure 4.9. There exists a significant rise in the HFD group noted. Meanwhile, the administration of garlic exosome to the HFD+Exosome I recorded the highest body weight level among other groups. Whereas administration of garlic exosome to the HFD+Exosome II, meaningfully reduced ($P < 0.05$) the rat's body weight.

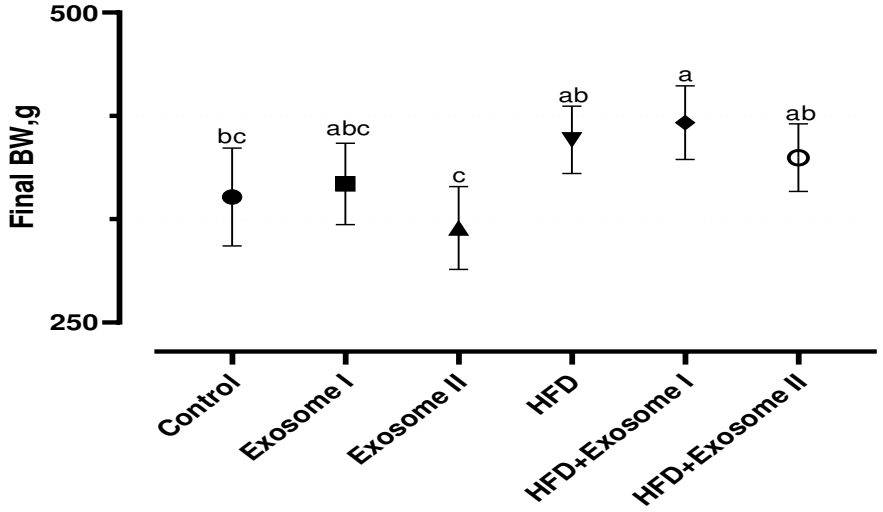

Figure 4.10. Effect of garlic exosome on final body weight. The statistical comparisons are shown by different superscripts (a-b) in the same row ($P < 0.05$; *ANOVA and Turkey's *post-hoc test*).

The highest body weight growth is in the HFD group and HFD+Exosome I group. However, garlic exosome administration to the HFD+Exosome II slightly reduced body weight but statistically not significant. Also, the rates of bodyweight growth between groups of control, Exosome I, and Exosome II display a slight difference but statistically insignificant. As shown in Figure 4.11.

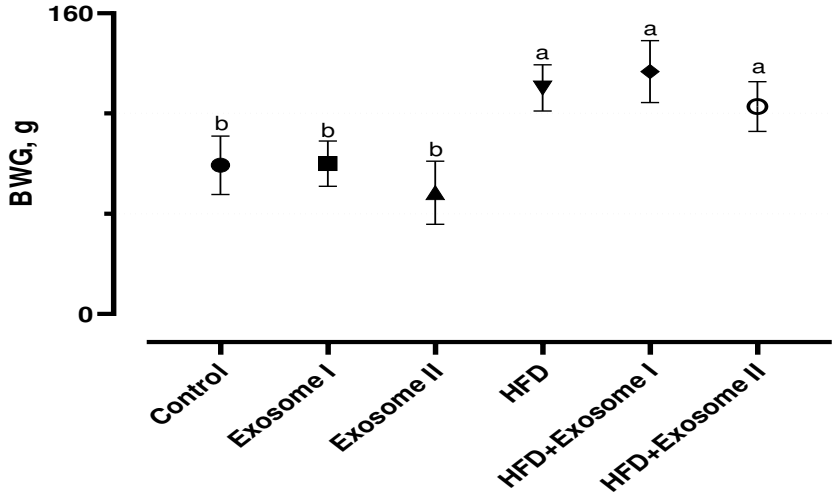

Figure 4.11. Effect of Garlic exosome on growing body weight of the rats

4.10. Effects of Garlic Exosome on Liver Tissues Histopathological Change

Control group

The sections prepared from the control group show the typical structure of the central vein and the usual radical arrangement of hepatocytes around the central vein with an average size of sinusoids and normal kupffer cells, as shown in Figure 4.12 and Figure 4.13.

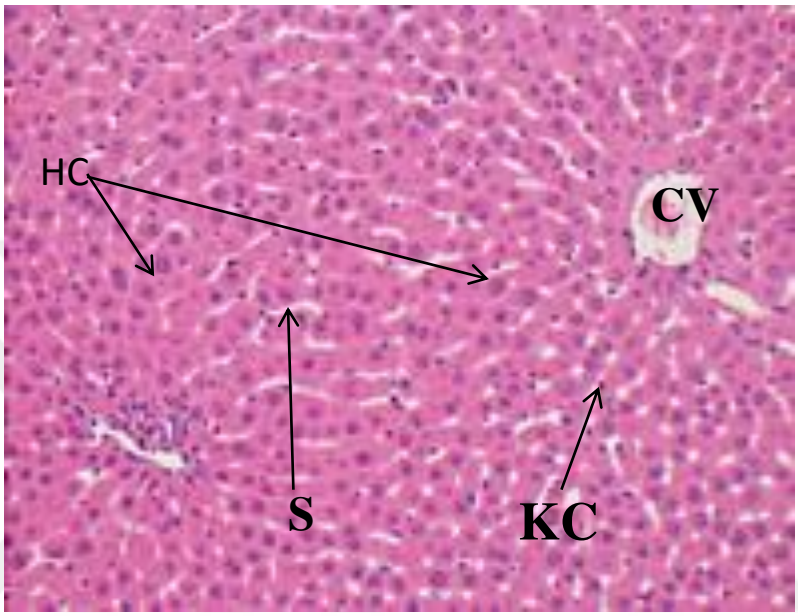

Figure 4.12. Liver of control group show normal structure of central vein (CV), sinusoids (S), hepatocytes (HC), and kupffur cells (KC) H&E.

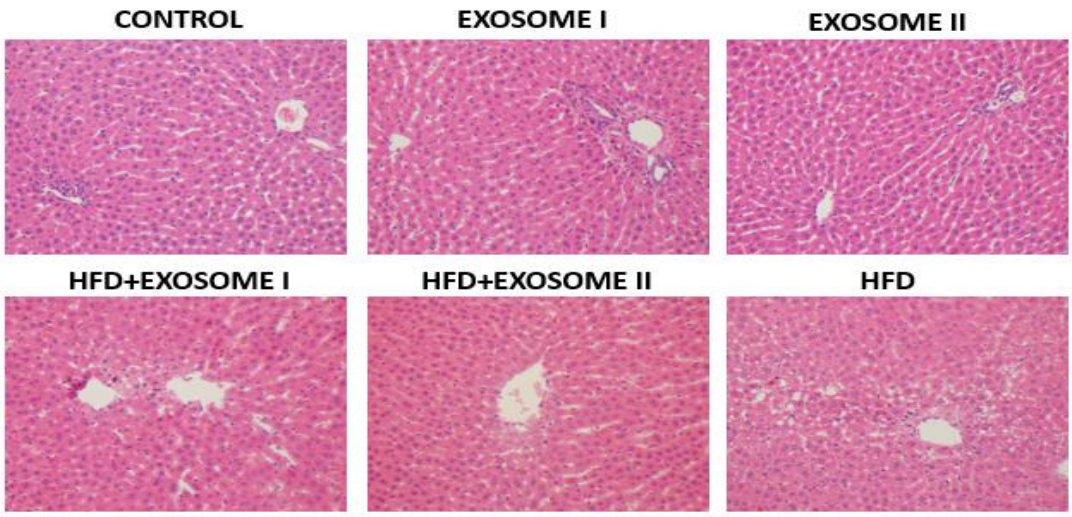

Figure 4.13. Effect of Garlic exosome on liver tissues histopathological alteration

4.10.1. Exosome groups

The sections prepared from Exosome groups (I & II) show the standard structure of the central vein and the typical radical arrangement of hepatocytes near the central vein with normal sinusoids, as shown in Figure 4.14a and Figure 4.14b.

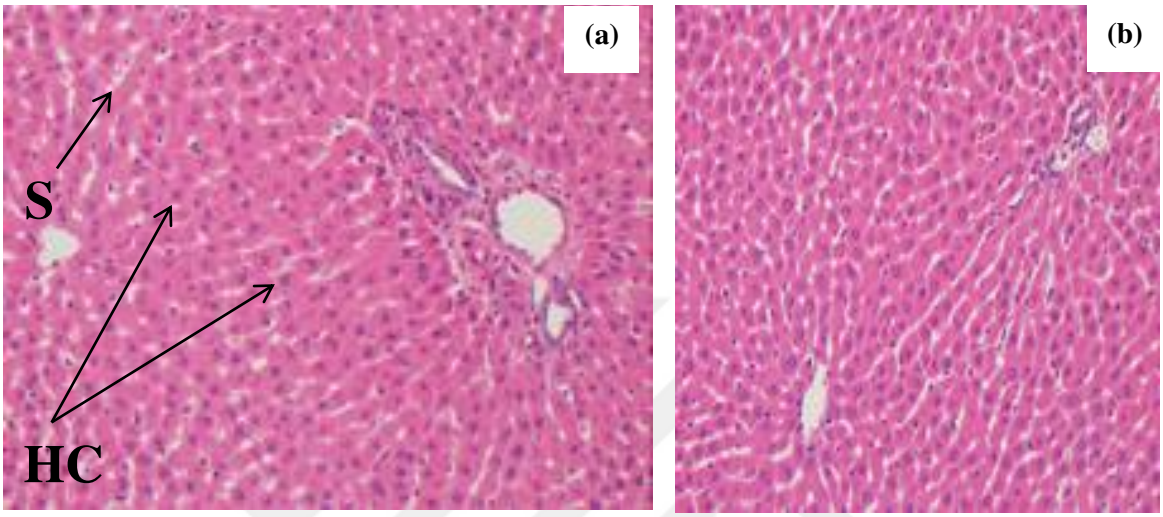

Figure 4.14. Liver of control group show normal structure of central vein (CV), hepatocytes (HC), sinusoids (S) and kupffur cells (KC) H&E.

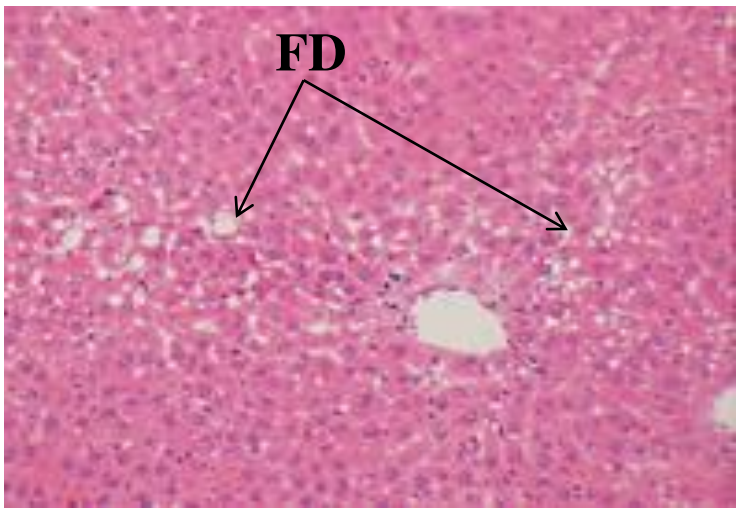

Figure 4.15. Liver of HFD group show lipid droplets (FD) and steatosis degeneration H&E.

4.10.2. HFD groups

Tissue sections prepared from this group show accumulation and deposition of large amounts of lipid droplets and steatosis degeneration in hepatocytes in most liver lobules, as shown in Figure 4.15.

4.10.3. HFD & Exosome groups

Tissue sections prepared from HFD & Exosome I group show fatty droplets continue to accumulate, but in minimal quantities, which means there is a slight improvement with edema, as shown in figure (4.12.D). The HFD & Exosome II group tissue slides showed a significant improvement in liver tissue, as the central veins and hepatocytes were semi-normal about the control group, as shown in Figure 4.16. Tissue sections prepared from this group show accumulation and deposition of large amounts of lipid droplets and steatosis degeneration in hepatocytes in most liver lobules, as shown in Figure 4.15.

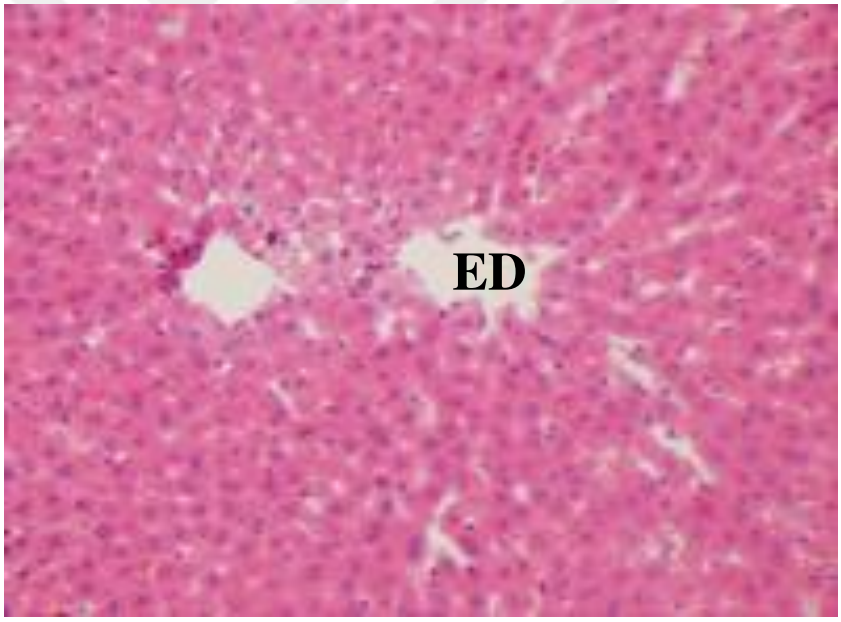

Figure 4.16. liver of HFD & Exosome I group show Edema (ED) H&E.

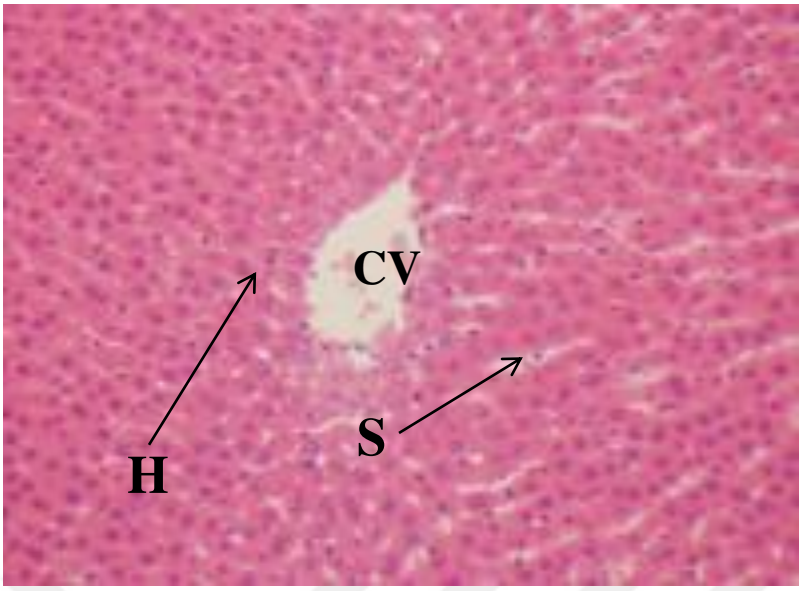

Figure 4.17. Liver of HFD & Exosome II group show of central vein (CV), hepatocytes (HC) and sinusoids (S) H&E..

4.11 Effects of Garlic Exosome on the Biochemical parameters

The glucose levels in this work show non-significant ($P<0.05$) difference between Exosome I (103.14 ± 3.98) and Exosome II (101.43 ± 3.60) groups compared with the control group (101.14 ± 5.21). In contrast, the glucose levels show a significant ($P<0.05$) increase between the HFD group (115.29 ± 5.31) compared with the control group. After treatment with Exosome, the glucose levels show improvement in HFD+Exosome I (109.43 ± 3.31) and HFD+Exosome II (104.43 ± 3.51) groups compared with the HFD group. However, there appears a significant rise ($P<0.05$) in both groups in relation to the controls, as shown in Table 4.1.

The bilirubin levels show a non-significant ($P<0.05$) difference between Exosome I (23.43 ± 2.88) and Exosome II (22.14 ± 2.79) groups compared with the control group (18.43 ± 2.82). In comparison, the bilirubin levels show a significant ($P<0.05$) increase between the HFD group (35.57 ± 4.04) compared with the control group. After treatment with Exosome, the bilirubin levels show improvement in HFD+Exosome I (30.14 ± 3.85) and HFD+Exosome II (26.14 ± 3.89) groups compared with the HFD group. However, there appears a significant rise ($P<0.05$) in both groups in relation to the controls, as in Table 4.1.

The Total protein levels show a non-significant ($P<0.05$) difference between Exosome I (8.76 ± 0.17) and Exosome II (8.63 ± 0.26) groups compared with the control group (8.44 ± 0.30). In contrast, the Total protein levels show a significant ($P<0.05$) increase between the HFD group (9.57 ± 0.29) compared with the control group. After treatment with Exosome, the Total protein levels show improvement in HFD+Exosome I (9.30 ± 0.19) and HFD+Exosome II (8.93 ±

0.28) groups compared with the HFD group. However, there appears a significant rise (P<0.05) in both groups in relation to the controls in Table 4.1.

The Albumin levels show a non-significant (P<0.05) difference between Exosome I (4.57 ± 0.26) and Exosome II (4.51 ± 0.24) groups compared with the control group (4.47 ± 0.30). In contrast, the Albumin levels show a significant (P<0.05) increase between the HFD group (5.54 ± 0.30) compared with the control group. After treatment with Exosome, the Albumin levels show improvement in HFD+Exosome I (5.37 ± 0.26) and HFD+Exosome II (5.00 ± 0.22) groups compared with the HFD group. However, there appears a significant rise (P<0.05) in both groups in relation to the controls in Table 4.1.

The Globulin levels show a non-significant (P<0.05) difference between Exosome I (2.40 ± 0.22) and Exosome II (2.36 ± 0.26) groups compared with the control group (2.30 ± 0.22). In contrast, the Globulin levels show a significant (P<0.05) increase between the HFD group (3.56 ± 0.30) compared with the control group. After treatment with Exosome, the Globulin levels show improvement in HFD+Exosome I (2.91 ± 0.24) and HFD+Exosome II (2.50 ± 0.26) groups compared with the HFD group. However, there appears a significant rise (P<0.05) in both groups in relation to the controls in Table 4.1. The A/G ratio show non-significant (P<0.05) difference between Exosome I (3.46 ± 0.14), Exosome II (3.47 ± 0.25), HFD (3.23 ± 0.42), HFD+Exosome I (3.09 ± 0.56), and HFD+Exosome II (3.56 ± 0.13) groups compared with control group (3.40 ± 0.20) group as shown in Table 4.1.

ALT levels show a non-significant (P<0.05) difference between Exosome I (33.04 ± 0.37) and Exosome II (33.03 ± 0.26) groups compared with the control group (32.40 ± 1.54). In comparison, ALT levels show a significant (P<0.05) increase between the HFD group (46.14 ± 2.38) compared with the control group. After treatment with Exosome, ALT levels show improvement in HFD+Exosome I (41.11 ± 1.70) and HFD+Exosome II (38.30 ± 1.97) groups compared with the HFD group. However, there appears a significant rise (P<0.05) in both groups in relation to the controls in Table 4.1.

AST levels show a non-significant (P<0.05) difference between Exosome I (103.71 ± 7.13) and Exosome II (102.29 ± 6.21) groups compared with the control group (103.43 ± 13.55). In contrast, AST levels show a significant (P<0.05) increase between the HFD group (201.00 ± 9.35) compared with the control group. After treatment with Exosome, AST levels show improvement in HFD+Exosome I (175.14 ± 21.33) and HFD+Exosome II (136.86 ± 16.15) groups compared with the HFD group. However, there appears a significant rise (P<0.05) in both groups in relation to the controls in Table 4.1.

The ALP ratio show non-significant (P<0.05) difference between Exosome I (122.94 ± 19.79), Exosome II (121.68 ± 9.63), HFD (121.31 ± 13.68), HFD+Exosome I (120.50 ± 9.96),

and HFD+Exosome II (122.57 ± 8.70) groups compared with control group (121.78 ± 17.82) group as shown in Table 4.1.

TBIL levels show non-significant ($P<0.05$) difference between Exosome I (0.22 ± 0.02) and Exosome II (0.23 ± 0.01) groups compared with control group (0.23 ± 0.02). Whereas TBIL levels show a significant ($P<0.05$) increase between the HFD group (0.28 ± 0.04) compared with the control group. After treatment with Exosome, TBIL levels show improvement in HFD+Exosome I (0.23 ± 0.03) and HFD+Exosome II (0.21 ± 0.03) groups than the HFD group and non-significant difference ($P<0.05$) in both groups than in the control group as shown in Table 4.1.

MDA levels show a non-significant ($P<0.05$) difference between Exosome I (0.91 ± 0.03) and Exosome II (0.64 ± 0.05) groups compared with the control group (0.66 ± 0.08). In contrast, MDA levels show a significant ($P<0.05$) increase between the HFD group (1.20 ± 0.07) compared with the control group. After treatment with Exosome, MDA levels show improvement in HFD+Exosome I (1.23 ± 0.07) and HFD+Exosome II (0.89 ± 0.10) groups compared with the HFD group. However, there appears a significant rise ($P<0.05$) in both groups in relation to the controls in Table 4.1.

Items	Groups					
	Control	**Exosome I**	**Exosome II**	**HFD**	**HFD+Exosome I**	**HFD+Exosome II**
GLU, (mg/dl)	101.14 ± 5.21^{c}	103.14 ± 3.98^{bc}	101.43 ± 3.60^{c}	115.29 ± 5.31^{a}	109.43 ± 3.31^{ab}	104.43 ± 3.51^{bc}
BUN, (mg/dl)	18.43 ± 2.82^{d}	23.43 ± 2.88^{cd}	22.14 ± 2.79^{cd}	35.57 ± 4.04^{a}	30.14 ± 3.85^{ab}	26.14 ± 3.89^{bc}
TP, (g/dl)	8.44 ± 0.30^{d}	8.76 ± 0.17^{cd}	8.63 ± 0.26^{cd}	9.57 ± 0.29^{a}	9.30 ± 0.19^{ab}	8.93 ± 0.28^{bc}
ALB, (g/dl)	4.47 ± 0.30^{c}	4.57 ± 0.26^{c}	4.51 ± 0.24^{c}	5.54 ± 0.30^{a}	5.37 ± 0.26^{ab}	5.00 ± 0.22^{b}
GLOB, (g/dl)	2.30 ± 0.22^{c}	2.40 ± 0.22^{c}	2.36 ± 0.26^{c}	3.56 ± 0.30^{a}	2.91 ± 0.24^{b}	2.50 ± 0.26^{c}
A/G	3.40 ± 0.20	3.46 ± 0.14	3.47 ± 0.25	3.23 ± 0.42	3.09 ± 0.56	3.56 ± 0.13
ALT, (U/L)	32.40 ± 1.54^{d}	33.04 ± 0.37^{d}	33.03 ± 0.26^{d}	46.14 ± 2.38^{a}	41.11 ± 1.70^{b}	38.30 ± 1.97^{c}
AST, (U/L)	103.43 ± 13.55^{d}	103.71 ± 7.13^{d}	102.29 ± 6.21^{d}	201.00 ± 9.35^{a}	175.14 ± 21.33^{b}	136.86 ± 16.15^{c}
ALP, (U/L)	121.78 ± 17.82	122.94 ± 19.79	121.68 ± 9.63	121.31 ± 13.68	120.50 ± 9.96	122.57 ± 8.70
TBIL, (mg/dl)	0.23 ± 0.02^{b}	0.22 ± 0.02^{b}	0.23 ± 0.01^{b}	0.28 ± 0.04^{a}	0.23 ± 0.03^{b}	0.21 ± 0.03^{b}
MDA, (umol/lt)	0.66 ± 0.08^{c}	0.91 ± 0.03^{b}	0.64 ± 0.05^{c}	1.20 ± 0.07^{a}	1.23 ± 0.07^{a}	0.89 ± 0.10^{b}

Table 4.1. Effects of Garlic *Exosome* on normal, HFD and diabetic rat biochemical parameters

HFD; High fat diet GLU; glucose BUN; bilirubin TP; total protein ALB; albumin GLOB; globulin A/G; albumin/ globulin ALT; Alanine aminotransferase AST; Aspartate Aminotransferase ALP; Alkaline Phosphatase TBIL; Total bilirubin MDA; Malondialdehyde ; ^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

5. DISCUSSION

In this chapter, further discuss the results and this thesis with comparison to other literature.

5.1. Body weight

The current work results revealed significant differences ($P<0.05$) at the end of the experiment, including an increase in the bodyweight of HFD and HFD+Exosome I&II groups, while there was a significant drop ($P<0.05$) in the weight of the Exosome I&II group compared to the control group. Epidemiological studies have shown a positive relationship between dietary fat intake and. Since rats and mice show a similar relationship, they are considered an appropriate model for studying dietary obesity. Epidemiological examinations have indicated a positive connection between dietary fat consumption and obesity. Because rats and mice show a similar trend, they are viewed as a fitting model for examining dietary stoutness [61]. The current study results agree with [62], who referred that HFD leads to obesity. In their work, they use twenty-four-week-old male rats and uninterruptedly fed a control diet (CD) or HFD for 51 weeks. The mean body weight of HFD-fed rats was higher than in control weights and markedly rose during the last 24 weeks. A high-fat diet typically encourages an elevated calorie intake and high energy density on increasing body weight, circulating serum lipid profile, elevated adipose tissue, hepatic steatosis, and glucose intolerance [63]. The factors causing obesity by a diet rich in fat involve a failure to adjust fat oxidation to more fat in the diet. The rise in adipose tissue lipoprotein lipase activity [64]. This is why there is a highly significant rise($p<0.001$) in the bodyweight of rats consuming high-fat diets.

5.2. Biochemical and histological study

The information in Table 4-1 demonstrates that there were no significant differences $P < 0.05$ in the albumin level of both groups, the control and the hypercholesterolemic. Low serum protein was seen in the HPC that took care of rodents and could be because of helpless feed consumption and usage. As a result of the energy thickness, the high-fat eating regimen may have prompted early satiety, diminished feed having, and disabled protein assimilation and different supplements [65].The significant purpose behind the checked lessening ($P < 0.05$) in serum glucose in the HPC took care of rats was dubious, yet it may be that the high serum cholesterol expands the degree of glucagon-like peptide-1, which upgrades insulin emission from the pancreatic beta-cells prompting hypoglycemia. It could likewise be because of diminished glucose stock through the eating regimen and resulting interest for pentose and glycogen

arrangement. Reports have demonstrated that immersed fats and polyunsaturated fats may cooperate to manage the outflow of a few compounds engaged with sugar digestion [66].

The current study results showed a significant ($P<0.05$) increase in liver function enzymes and bilirubin levels in the HFD group compared with the control group. The current findings agree with the study of [87], who referred that hyperlipidemic patients suffering from increased ALT and AST compared with healthy persons. In a study carried out by Zhang et al. (2020), there were improved profiles of serum ALT and AST consistent with enhanced serum cholesterol levels in HFHC-fed mice. They offer liver histology of HFHC-fed mice with steatosis with mild inflammation, steatohepatitis with fibrosis, confirming current study findings. Also, the histological findings were similar to our biochemical findings. The use of hyperlipidemia in rats demonstrated extreme greasy changes in liver hepatocytes. This impact might be attributed to the amassing and statement of bountiful fat beads in hepatocytes, which involved the whole-cell cytoplasm [67, 68]. Results of the histology of the liver showed different degrees of histological changes due to a large number of histological changes in different study groups. Therefore, our classification of these changes in the liver tissue consists of five scores. The most severe changes were observed in hyperlipidemic positive control rats. These changes included: large numbers of the micro, middle, and macro-vesicular steatosis in the liver tissue. Our diet model consists of cholic acid; this material is well known to aggravate induce hyperlipidemia by two mechanisms of action: an increase in cholesterol absorption in the mucosal intestine and concomitant suppression of cholesterol 7α-hydroxylase activity, a key enzyme in the structure of the bile acids from cholesterol decreasing cholesterol excretion. Also, cholic acid improves cholesterol absorption with its emulsifying features [69].

Liver injury in HF diet-fed rats results from increased production of peroxides or increased sensitivity to peroxides due to cross-talk between cholesterol and selenoprotein biosynthesis pathways. Injury to liver tissues due to hyperlipidemia alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. Therefore, the marked release of ASAT, ALAT, and GGT into the circulation indicates severe damage to hepatic tissue membranes [45], which agrees with the current study findings. The enzyme activities shown in Table 4-1 indicated that AST and ALT were elevated in the serum of the HPC-fed rats. This could result from leakage of the enzymes into the serum as a result of damage to the integrity of the heart and liver. Elevated serum activity of these enzymes has been reported to indicate the calculated risk of cardiovascular disease. According to Pincus, AST and ALT are released into serum when there is severe hepatocellular injury. Liver sections in most severe cases revealed different degrees of hepatocytes degeneration, pyknotic nuclei, all of which refer to cell death

during induction of hyperlipidema. These changes probably due to an increase of oxygen free radicals, which are reported to be generated during hypercholestrolemic atherogenesis. Thus, Oxidative modification of lipid, i.e., oxidized low-density lipoprotein (Ox-LDL), has been suggested to play a critical role in the pathogenesis of atherosclerosis; LDL oxidation plays a vital role in plaque growth degenerative changes leading to cell disruption and death. The results of the current study agreed with the results, who referred that a high-fat diet caused the high concentration of cholesterol and liver histological changes due to defect in the process of fat metabolism, or a defect in the process of absorption and subtraction of steroids or perhaps because of low concentration of bile salts.

The role of exosomes that extract from a plant in this study shows the vital role of exosomes in treating liver damage induced by a high-fat diet. The present findings agree with the study of [70], who referred that plant exosomes have been shown to protect the body from alcohol-induced liver damage. Lipid analysis showed that plant exosomes are primarily enriched in phosphatidic acids. Localization of plant exosomes in vivo showed that these particles localize mainly in the liver and lymph nodes in 12 hours. administration of plant exosomes to these animals promoted decreased lipid droplets in the liver and triglyceride levels. Consequently, a combination of these results showed that plant exosomes could protect mice from alcohol-induced liver damage.

The present work has shown that hypercholesterolemia was linked with a significant rise in MDA serum concentration. This rise in rabbits consumed high cholesterol diet confirms many experimental studies [71]. Their clinical research argues that the MDA serum concentration of hyperlipidemic subjects is considerably higher than that of hypolipidemic groups. The free radical attack initiated lipid peroxidation on membrane polyunsaturated fatty acids 1 causing their fragmentation and transformation into alkanes and reactive aldehyde compounds. MDA is the most commonly used lipid peroxidation indicator; it measures free radical generation and an elevated level of the same in high-fat diet rabbits. This means that hypercholesterolemia may raise the lipid peroxidation process [55].

In the current study, hypercholesterolemia was associated with a significant increase in serum concentration of MDA along the study time. The observed increase in MDA level in rabbits fed high cholesterol diet is consistent with several experimental studies [72] reported in their clinical study that serum concentration of MDA of hyperlipidemic subjects is significantly higher than that of hypolipidemic ones. Lipid peroxidation was initiated by the free radical attack on membrane polyunsaturated fatty acids leading to their transformation and fragmentation to alkanes and reactive aldehyde compounds. MDA is a product and the most frequently used indicator of lipid peroxidation; it is a measure of free radical generation. An elevated level of the

same in high-fat diet rabbits suggests that hypercholesterolemia could enhance the process of lipid peroxidation [55]. About the antioxidant activity of exosomes in the present study, several studies showed ginger, grapefruit, and broccoli exosomes can increase antioxidant and anti-inflammatory mediators in macrophages while limiting the production of proinflammatory cytokines such as TNF-α and IL-1β. However, the molecular mechanisms underlying these effects are not known. Recently, nine fruit and vegetables were tested for the activity of their exosome-like NVs in respect to the nucleotide-binding domain and leucine-rich repeat-containing family, pyrin domain containing 3 (NLRP3) inflammasome.

Lumens of exosomes contain mRNA, proteins, microRNAs (miRNAs), and long noncoding RNAs. Evidence suggests that mRNAs in target cells can translate into functional proteins. Diet/plant-derived miRNAs described above seem to act in a hormone-like fashion. The miR-335 was indicated to modulate immune responses during the unidirectional transfer from T cells to antigen-presenting cells (APC). These results highly suggest that secreted miRNAs represent a novel mode of signaling for long-distance transportation of messages, by which donor cells can influence gene expression of recipient cells, and thus impact physiological and pathological processes that explain the role of exosomes and their effect on leukocytes counts and activity in the current study. The mechanism of exosomes uptake influences the destination of their contents. Uptake by clathrin-mediated endocytosis (i.e., receptor-mediated endocytosis), caveolin-mediated endocytosis, and macropinocytosis result in the delivery of exosomes and their contents into early endosomes. From there, EVs and their contents may be released into the cytoplasm or be funneled into the lysosome pathway for degradation. Exosomes can also be internalized by phagocytosis, resulting in their delivery into phagosomes. Alternatively, exosomes may fuse directly with the plasma membrane, delivering their contents directly into the cytoplasm of the recipient cell.

6. CONCLUSIONS

The results show an increase in body weight after using a high-fat diet and Exosome I&II.

a. Glucose, bilirubin, Total Protein, Albumin, Globulin, A/G ratio, liver enzymes, TBIL, MDA levels show significant ($P<0.05$) increase after using a high-fat diet.
b. Glucose, bilirubin, Total Protein, Albumin, Globulin, A/G ratio, liver enzymes, TBIL, MDA levels show non-significant ($P<0.05$) increase after using Exosome I&II.
c. AP2 protein, LPL protein, NFκB protein, Nrf2 protein, PPAR-α protein, SREBP-1c protein, and TNF-α protein levels show significant ($P<0.05$) increase after using a high-fat diet and Exosome I&II.
d. HO1 protein shows a non-significant ($P<0.05$) increase after using Exosome I&II.
e. Histological study shows the typical structure of liver tissues after using Exosome I&II. While after using a high-fat diet, liver tissues show accumulation and deposition of large amounts of lipid droplets and steatosis degeneration in hepatocytes in most lobules.

RECOMMENDATIONS

For further investigation of those interested in this study on the protective role of exosomes on the clinical disorders in obese mouse models affected by the accumulation of fat in the liver leading to many diseases, we suggest adding another group of rats and increasing the dose of the exosome. This step will help the researcher to determine the effect of the exosome on the liver fat.

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APPENDİCES

APPENDIX- 1: LAB WORK

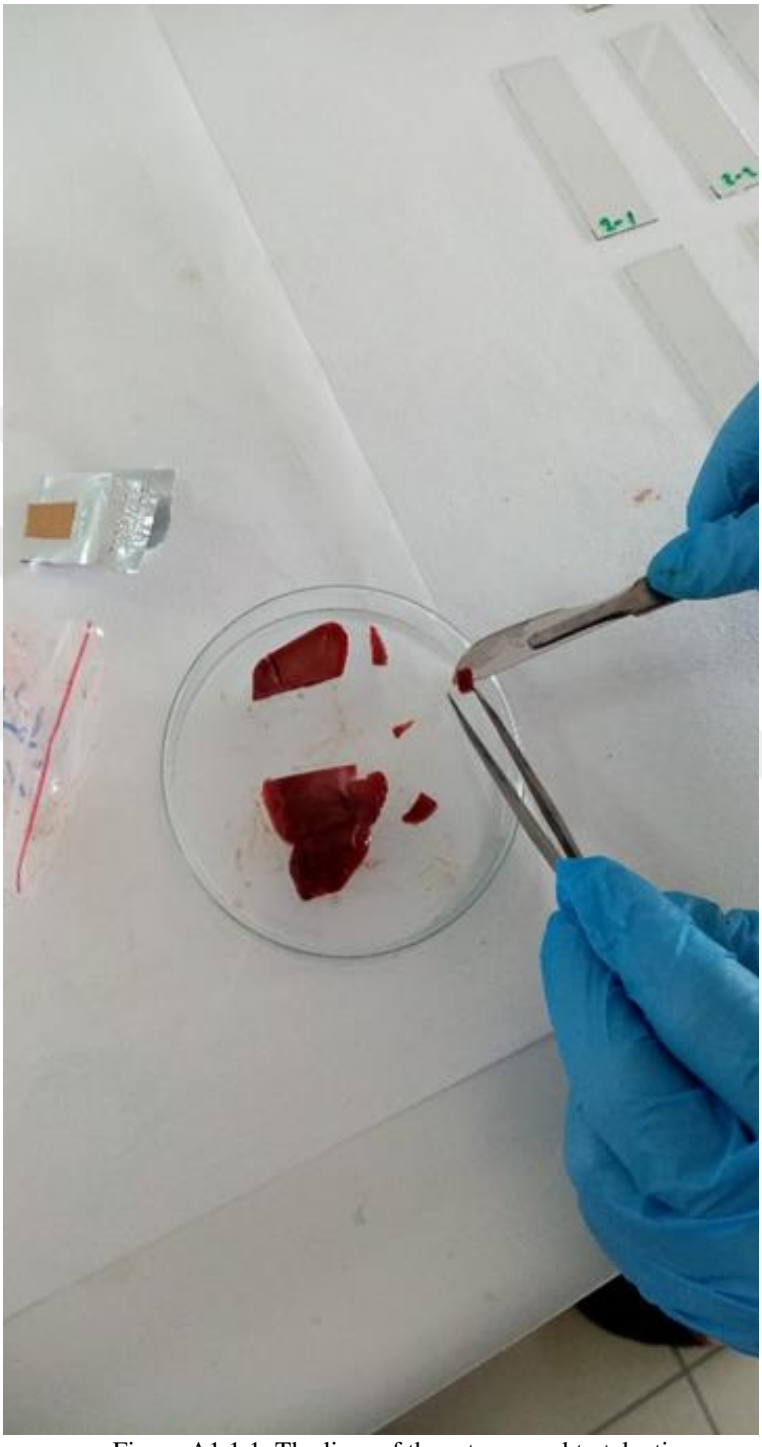


Figure A1.1 1. The liver of the rats opened to take tissues

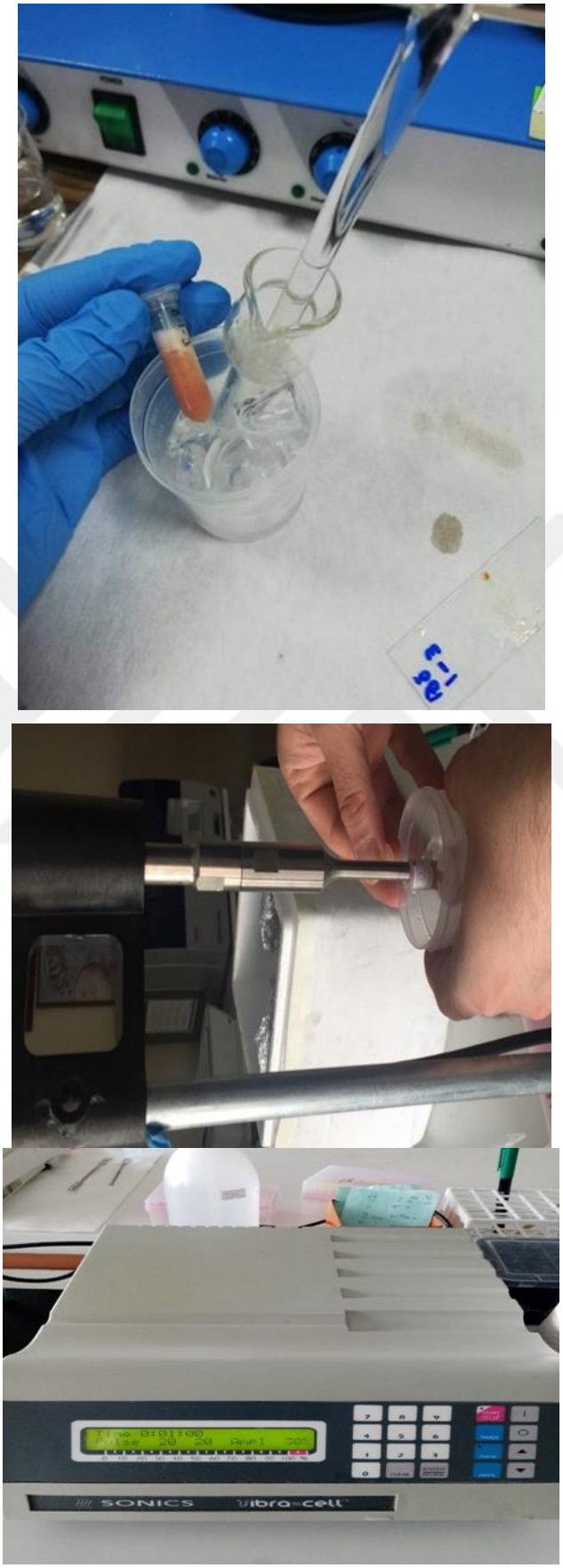


Figure A1.1 2. . Liver tissue homogenization process

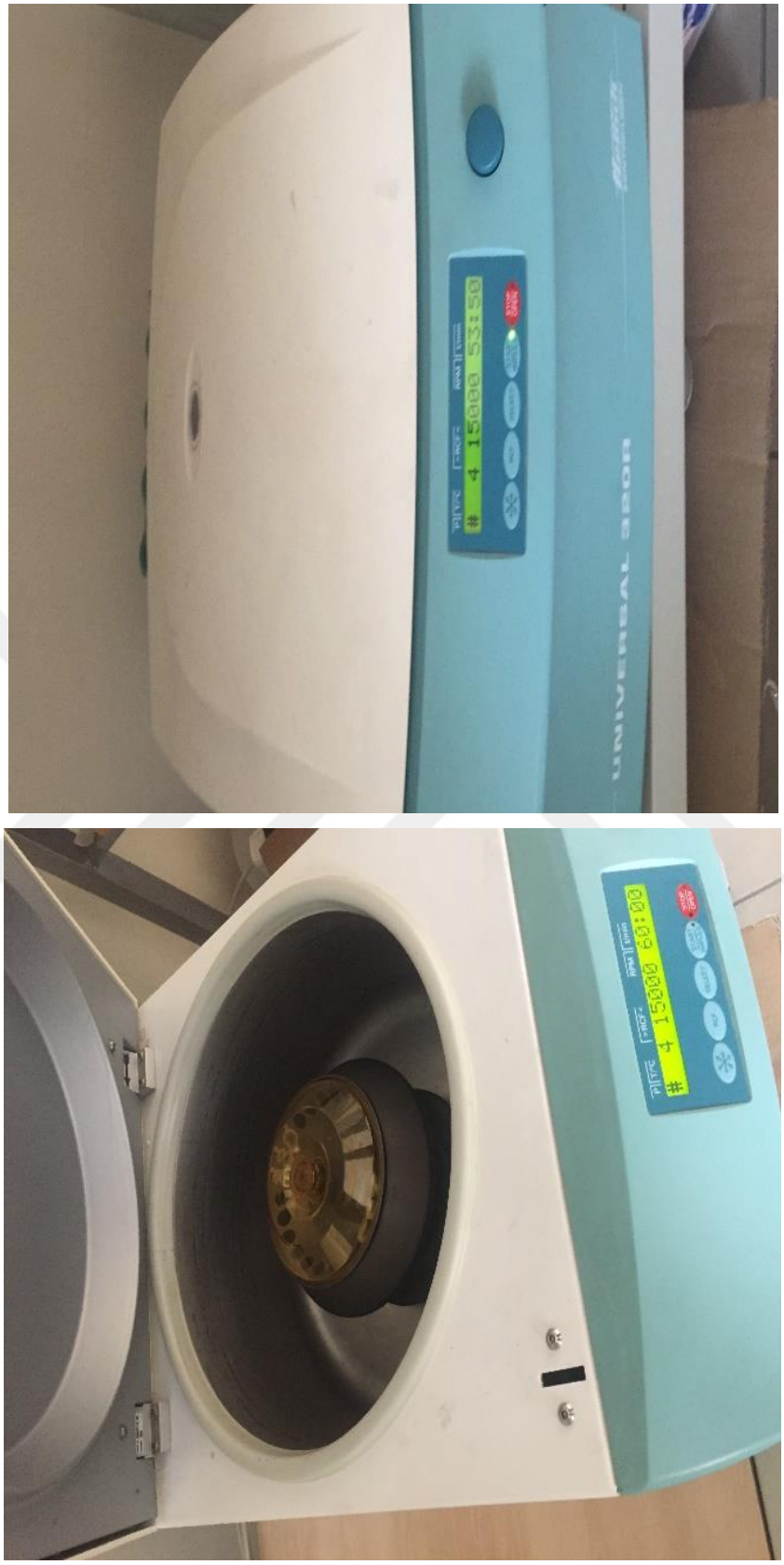
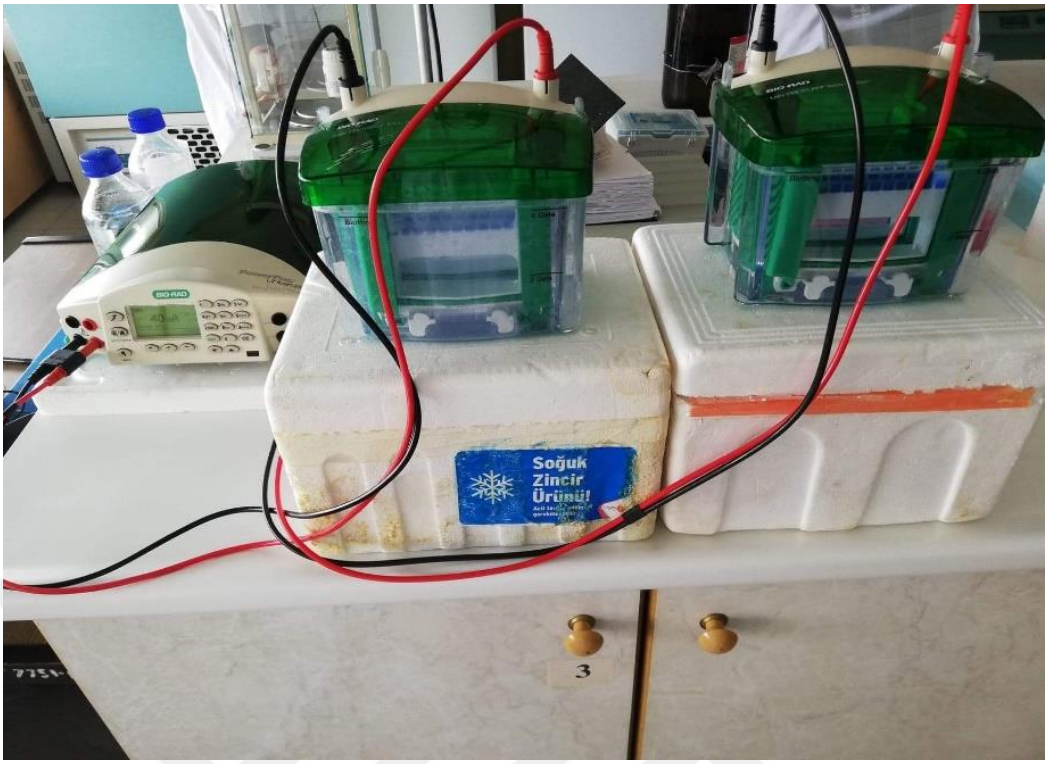


Figure A1.1 3. Centrifugation of homogenized liver tissues to obtains its supernatant



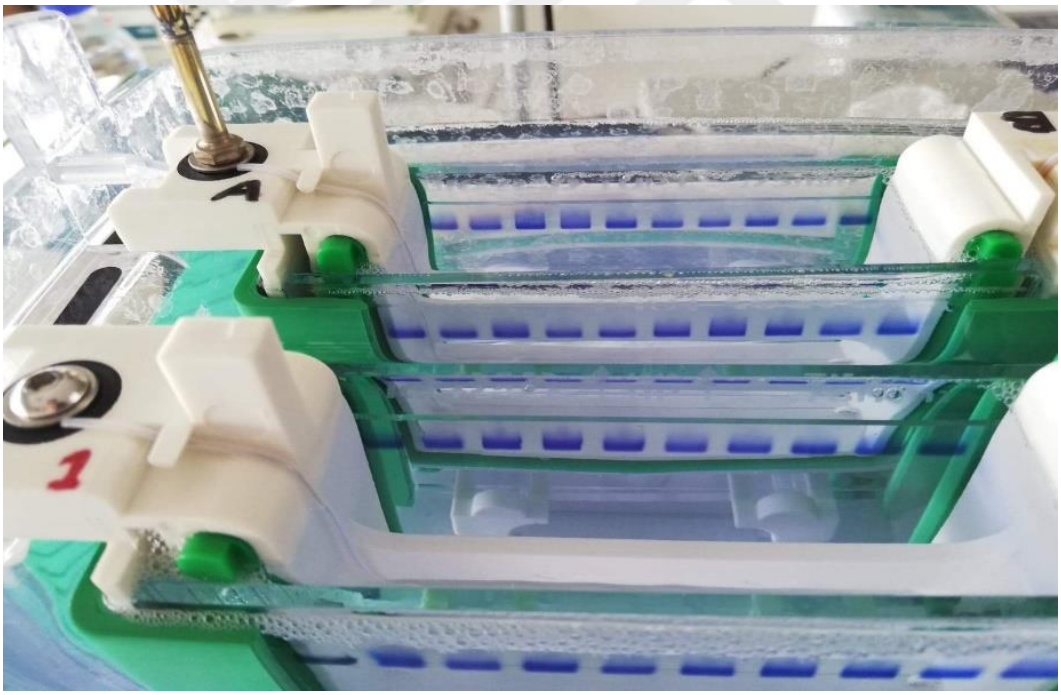


Figure A1.1 4. Protein samples loading into the gels and running the proteins

Figure A1.1 5. **T**ransportation of protein bands from the gels on to the nitrocellulose membranes

Figure A1.1 6. Incubation of the nitrocelluloses membranes in bovine serum albumin

CURRICULUM VITAE

Diyar Ali MAHMOOD

[redacted]

[redacted]

[redacted]

RESEARCH EXPERIENCES

- ✓ Laboratory Instruments you use, such as test systems etc. you know
- ✓ Computer Programming languages available (C-C++, MATLAP, LABVIEW, vb.)
- ✓ Computer programs you can use (CAT/CAM, PROTEUS, CALCULUS, vb.)

WORK EXPERIENCE

2013 – 2021: Teacher in high school. Kirkuk/Iraq

ACADEMIC ACTIVITIES

- ✓ Biology teacher in high school. Kirkuk/Iraq
- ✓ Graduate research (BA) on skin cancer.