NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY

HISTOLOGICAL, BIOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY OF ZINGIBER OFFICINALE ON EXPERIMENTALLY INDUCED FATTY LIVER IN RAT

(M.Sc. THESIS)

HAREZ JAWDAT JAAFAR

NICOSIA

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SUPERVISOR

Prof. Dr. Aysel KÜKNER

CO-ADVISOR

Asst. Prof. Dr. Twana A Mustafa

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Declaration

I declare that this thesis is my own work, that I do not engage in unethical behavior at all stages from the planning to the writing of the thesis, that I have obtained all the information in this thesis within the framework of academic and ethical rules, that I have cited all the information and comments that were not obtained with this thesis study, and that I have included these sources in the references list, I declare that I have not acted in violation of patents and copyrights during the thesis work and writing process.

Name and Surname: Harez Jawdat JAAFAR

Date:

Signature:

Acknowledgement

I would like to express all my respect and gratitude to my thesis supervisor Prof. Dr. Aysel KÜKNER and co-advisor Asst. Prof. Dr. Twana A Mustafa for their greatest inspiration to me and their continuous support with their knowledge, patience and motivation at every step of my M.Sc.

Above all, I would like to express my endless thanks and love to my beloved family for their loyalty and great trust in me. I owe a lot to my father Prof. Dr. Jawdat Jaafar. I would like to thank my mother, sisters and brothers for supporting, encouraging and giving me constant love throughout my life.

Finally, I have a long list of friends I want to thank. I cannot mention all of them, but I would like to thank them wholeheartedly for their valuable help and support since my first work.

Name and Surname: Harez Jawdat JAAFAR

Abstract

Histological, Biochemical and Immunohistochemical Study of *Zingiber officinale* on Experimentally Induced Fatty Liver in Rat

Harez Jawdat Jaafar

Supervisors:

Prof. Dr. Aysel KÜKNER - Department of Histology and Embryology

Asst. Prof. Dr. Twana A Mustafa – Department of Medical Laboratory Techniques

AIM: The aim of this study is to investigate the histological, biochemical and immunohistochemical potential protective effects of *Zingiber officinale* (ginger) against liver steatosis and high-fat diet induced dyslipidemia.

METHODS: Thirty male adult Wistar albino rats were used in this study; these were divided randomly to five equal groups. The animals were fed on standard diet for 5 weeks (group I), high-fat diet group (group II), standard diet containing ginger extract "200mg/kg" (group III), protective group, subdivided into two equal subgroups, HFD containing ginger "200 mg/kg" (group IV), and standard diet with ginger extract for 5 weeks then stopped and shifted to high-fat diet for additional 5 weeks (group V).

RESULTS: Significantly ($p<0.05$) elevated total cholesterol, triglyceride, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and alkaline phosphatase levels were observed among rats in the high-fat diet (group II) as compared to control groups. The group received 200 mg of ginger extract (group III) revealed a statistically significant ($p<0.05$) increase in alkaline phosphatase, SGOT and SGPT. The results of the group IV and V that induced fatty liver plus ginger, revealed statistically significant ($p<0.05$) decrease of alkaline phosphatase, with, significant ($p<0.05$) decrease of cholesterol, triglyceride, SGOT and SGPT levels, whereas, non-significant ($p<0.05$) decrease of HDL and LDL levels revealed among the group that

induced fatty liver plus ginger extract compared with fatty liver group. Examination of the liver of the HFD-fed rats showed many pathological changes, as compared with the control. However, examination of liver of animals fed on HFD along with ginger showed a remarkable improvement especially after 5 weeks; the histological pictures were mostly similar to the control group.

CONCLUSION: Ginger has a protective role against developing nonalcoholic fatty liver and improving the lipid profile. Therefore, according to the data we found ginger ingestion is safe in humans and might be a promising hepatoprotective agent.

KEYWORDS: Zingiber officinale, ginger, fatty liver, rat

Özet

Histological, Biochemical and Immunohistochemical Study of *Zingiber officinale* on Experimentally Induced Fatty Liver in Adult Male Albino Rat

Harez Jawdat Jaafar

Danışmanlar:

Histoloji ve Embriyoloji Anabilim Dalı - Prof. Dr. Aysel KÜKNER

Tıbbi Laboratuvar Teknikleri Anabilim Dalı - Asst. Prof. Dr. Twana A Mustafa

AMAÇ: Bu çalışmada Zingiber officinale'nin yüksek yağlı diyete bağlı dislipidemi ve karaciğer yağlanmasına karşı histolojik, biyokimyasal ve immünohistokimyasal potansiyel koruyucu etkisi araştırıldı.

YÖNTEMLER: Çalışmada 30 yetişkin erkek Wistar albino sıçanı kullanıldı, denekler rastgele beş gruba ayrıldı. Gruplar; Standart diyet ile beş hafta boyunca beslenen kontrol grubu (grup I), Yüksek yağlı diyet grubu (grup II), 200 mg/kg zencefil özü içeren standart diyet grubu (grup III), yüksek yağlı diyet + zencefil "200 mg/kg" verilen grup (grup IV) ve 5 hafta boyunca zencefil suyu + standart diyet ile beslemek ve daha sonra bu diyet kesilerek 5 hafta daha yüksek yağlı diyet ile beslenen grup (grup V) olarak düzenlendi.

BULGULAR: Total kolesterol, trigliserit, Serum Glutamik Oksaloasetik Transaminaz (SGOT), Serum Glutamik Piruvik Transaminaz (SGPT) ve alkalin fosfataz seviyelerinin yüksek yağlı diyet grubunda (grup II), kontrol gruplarına göre anlamlı olarak yükseldiği ($p<0.05$) gözlendi. 200 mg/kg zencefil özü ile beslenen grupta (grup III), alkalin fosfataz, SGOT ve SGPT düzeylerindeki artış istatistiksel olarak anlamlı ($p<0.05$) bulundu. Grup IV ve V'de, kolesterol, trigliserit, SGOT, ve SGPT düzeylerindeki anlamlı ($p<0.05$) düşüş alkalin fosfatazda istatistiksel olarak anlamlı ($p<0.05$) düşüş gösterdi, bununla birlikte,

yağlı karaciğer grubuna göre yağlı karaciğer + zencefil ekstraktını ile beslenen grupta HDL ve LDL düzeylerinde anlamlı ($p<0.05$) azalma saptandı. Kontrol grubu ile HFD ile beslenen sıçanlarım karaciğer dokuları karşılaştırıldığında, birçok histoloji değişiklik tespit edildi.

SONUÇ: Zencefilin non alkolik karaciğer yağlanmasına ve lipid profilini iyileştirmeye karşı koruyucu rolü olduğu görüldü. Bu nedenle, elde edilen verilere göre zencefil alımı insanlarda güvenlidir ve umut verici bir hepatoprotektif ajan olabilir.

ANAHTAR KELİMELER: Zingiber officinale, zencefil, yağlı karaciğer, sıçan

Table of Content

CHAPTER I

CHAPTER II

List of Tables

List of Figures

List of Histograms

List of Abbreviations

Acetyl CoA	Acetyl Coenzyme A
ALD	Alcoholic Liver Disease
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CCl4	Carbon Tetrachloride
CO2	Carbon Dioxide
CVD	Cardiovascular Diseases
DILI	Drug-Induced Liver Injuries
GFAP	Glial Fibrillary Acidic Protein
GS	Glomerulosclerosis
H&E	Hematoxylin and Eosin
HCC	Hepatocellular Carcinoma
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein Cholesterol
HFD	High-fat Diet
HMG-CoA	β-Hydroxy β-methylglutaryl-CoA
IHC	Immunohistochemistry
IL-6	Interleukin-6
LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein Cholesterol
NAFLD	Nonalcoholic Fatty Liver Disease
NASH	Nonalcoholic Steatohepatitis
N-CAM	Neural Cell Adhesion Molecule
PAS	Periodic Acid Schiff

RER	Rough Endoplasmic Reticulum
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SD	Standard Deviation
SER	Smooth Endoplasmic Reticulum
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
TC	Total Cholesterol
TG	Triglyceride
TNF-α	Tumor Necrosis Factor α
VLDL	Very Low Density Lipoproteins
Z. officinale	*Zingiber officinale*

CHAPTER I

Introduction

Ginger is the rhizome of the plant which is known as *Zingiber officinale* (*Z. officinale*) Roscoe which is belonging to the genus *Zingiber* and the Zingiberaceae family, it has long been used as an herbal medicine and spice (Baliga, 2012; Han, 2013). Powder, syrup, volatile oil and oleoresin can be processed from the ginger rhizome. The usage of ginger in culinary is dates back to 13th century (Langner, 1998). The plant is currently cultivated in India, the West Indies, Africa and other tropical regions, but is native to Asia. The root of ginger is used to relieve and treat many diseases such as pain, nausea, headache, vomiting and colds. Ginger includes (50 genera and 1600 species). The genus *Zingiber* includes 152 species, however, for flavoring ginger is the only species that is commonly used (Ravindran & Nirmal Babu, 2005). Grows between April and December at an optimum altitude of between 300 and 900 meters (Pruthy, 1993), prefers light shade and requires a warm, humid climate (Jayachandran, et al., 1991). Ginger has been cultivated in southern Asian countries for more than 3,000 years, and its discovery and value as a medicinal herb and spice is well documented. One of the earliest data about the ginger was made by Rabbi Benjamin Tudela while he was traveling between 1159 and 1173 and described the trade and cultivation of spices from the ancient port of Quilon in Kerala State (Mahindru, 1982). Other additional document dates back to 1298 A.D. was found in the travelogue of Marco Polo which indicates "good ginger grows here and is known by the name of Quilon ginger". Ginger contains many identified bioactive compounds for example, phenolics and terpene compounds as well. Phenolic compounds are usually gingerols, shogaols and paradol (Stoner, 2013). In recent years, it has been found that ginger has many common biological activities, for example, anti-inflammation (Zhang, 2016), antioxidant (Nile, 2015), antimicrobial (Kumar, 2014) and anticancer (Citronberg, 2013) properties. Also, accumulating researches have shown that ginger has the ability to manage and prevent various diseases, for example, cardiovascular diseases (Akinyemi, 2015), neurodegenerative diseases (Ho, 2013), obesity (Suk, 2017), diabetes mellitus (Wei, 2017), nausea and vomiting that induced from chemotherapy (Walstab, 2013), and some respiratory disorders (Townsend, 2013).

The chemical components of ginger rhizomes can vary significantly, according to the rhizome is freshly dried or processed, and also by location of cultivation (Ali, et al., 2008). The pungency of the fresh ginger is because of the groups of phenols and gingerols. Fresh ginger may contain a 5-deoxy derivative which is called paradol. The dry ginger exhibits sharpness due to shogaols, anhydrous forms of gingerol that result from thermal processing. Ginger also contains approximately 1% to 3% essential oils, which gives ginger a distinguish odor composed mostly of sesquiterpenoids and monoterpenoids, including zingiberene, camphene, sesquierpenoids, borneol and bisabolene (Chubrasik, et al., 2005: Ali, et al., 2008).

According to a recent report, that healthy people taking ginger in oral doses ranging from 100 mg to 2 grams showed that the main shogaol and gingerol components are easily absorbed and appear predominantly in serum as glucuronide conjugates, and at the same time no free forms were detected (Zick, et al., 2018). In another study, products of [6]-shogaol and [6]-gingerol and the degradation kinetics was characterized in a stomach and intestinal environment model under various physiological conditions (Bhattaraj, et al., 2007). Also there is evidence that the metabolism of [6]-gingerol, particularly by the enzymes in the liver of rats, as well as the metabolism of intestinal microorganisms, can influence the distribution of ginger constituent (Nakazawa, et al., 2002). In addition, preclinical researches with laboratory animals have demonstrated that ginger also has hepatoprotective effects and protects the liver against the toxic effects of various classes of xenobiotic agents such as, alcohol (Mallikarjuna, et al., 2008; Shati, et al., 2009), acetaminophen (Ajith, et al., 2007), heavy metals (Vitalis, et al., 2007), paraben (Verma, et al., 2007), carbon tetrachloride (CCl4) (Yemitan, et al., 2006), bromobenzene (El-Sharaky, et al., 2009). Supercritical carbon dioxide (CO2) extraction and steam distillation yields a volatile oil containing volatile components, while, extraction of solvents yield oleoresin containing tastants and non-volatiles (Ravindran & Nirmal Babu, 2005). Some of the major volatiles identified by Connell in 1970 include, sesquiterpene hydrocarbons such as (α-zingiberene, β-bisabolene, ar-curcumene, β-sesquiphellandrene, β-elemene, farnesene, γ-selinene and β-zingiberene). Other identified monoterpene hydrocarbons (α-pinene, β-pinene, limonene, cumene, β-phellandrene) and oxygenated compounds such as (1, 8-cineole, linalool, neral, d-borneol, geranial) (Connell, 1970). In 1917, Lapworth were first identified [6]-gingerol and in the 1969 Connell and Sutherland recognized and founded S-

configuration of the hydroxyl groups (Figure 1). Because of the thermally labile beta-hydroxy-keto group, gingerols are easily dehydrated and then form the corresponding shogaols (Figure 2).

Figure 1

S-[6]-gingerol

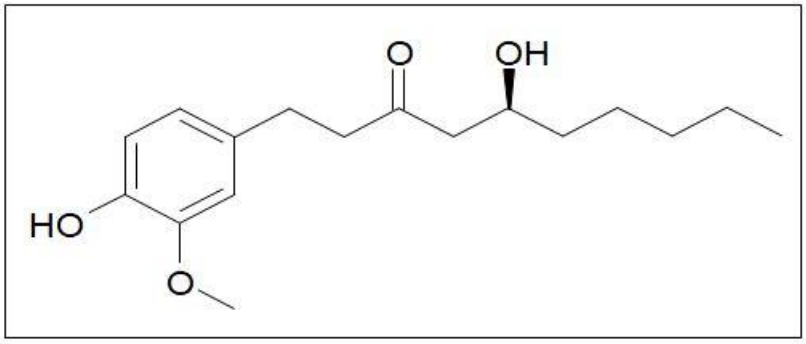
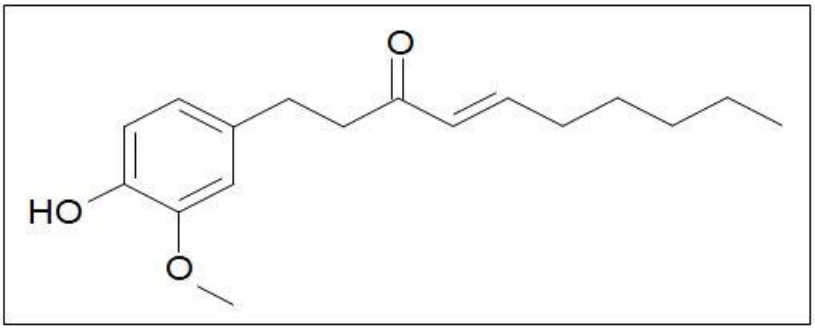


Figure 2

[6]-shogaol



After alkaline hydrolysis of gingerol, the production of n-hexanal gave the name [6]-gingerol, also, the name shogaol is derived from the Japanese word "shoga" meaning ginger. Gingerols and shogaols not responsible only for the pungency (bitterness of flavor and odor) of the ginger essential oil, it is also proven to be responsible for the antioxidant property of ginger (Kikuzaki & Nakatani, 1993).

1. GENERAL INFORMATIONS OF GINGER

1.1 Traditional Uses and Current Uses of Ginger

1.1.1 Use of Ginger as a Spice and Flavorings

According to the legend ginger bread was first made by a baker in Greece on the Isle of Rhodes around 2400 B.C. Gingerbread was known as Queen Elizabeth 1's favorite food in the 1500s, and throughout the Middle Ages, tavern operators they were constantly providing ground ginger powder so that the customers could use it on beer as described by Rosengarten in "The book of spices" (Rosengarten, 1969). Today ginger is used in many products such as, ginger ale, pumpkin pie spice, and Indian masala mixes. Products which contain ginger are more popular in Australia, Thailand, Japan and chine compared to Western world (Ravindran & Nirmal Babu, 2005).

1.1.2 Medicinal Purpose

Ginger is traditionally is used for the treatment of several diseases in dried and fresh forms in Indian, Chinese, Japanese and Indonesian medicines such as, asthma, rheumatism, arthritis, sore throats, motion sickness, muscular aches, sprains, vomiting, nausea, constipation, diarrhea and hypertension (Cho, et al., 2001; Badreldin, et al., 2008). In ancient times in India, ginger were mainly used as medicine rather than flavoring and It was called maha oushadha (great medicine) and vishwa bheshaja (universal cure) (Ravindran & Nirmal Babu, 2005). The modern homeopathic uses of ginger are very similar and are mostly used to treat pregnancy and motion sickness.

1.2 The Hepatoprotective Effects of Ginger

1.2.1 Drug-Induced Toxic Hepatitis and Liver Injury

In the last two decades the hepatoprotective effect of ginger extract has been demonstrated in rat studies on several chemical and Drug Induced Liver Injuries (DILI) with histopathological and serum biochemical evidences (Yemitan & Izebgu, 2006; Ajith, et al., 2007; Baiomy & Mansour, 2016; Essawy, et al., 2018; Badawi, 2019).

1.2.2 Alcoholic Liver Disease

According to biochemical data and histopathological examinations, ginger has a protective role against fatty liver disease and alcoholic liver disease (Liu, et al., 2013; Nwozo, et al., 2014). After alcohol administration, metabolomic data showed that the amounts of metabolites such as glycerol-3-phosphate, lithocholic acid, pyruvic acid and prostaglandin E1 increased, but the levels of rats in the ginger treatment group returned to normal (Liu, et al., 2013).

1.2.3 Nonalcoholic Fatty Liver Disease

Currently Nonalcoholic Fatty Liver Disease (NAFLD) is the most common liver disease, and in serious cases it can turn to cirrhosis, hepatocellular carcinoma and liver fibrosis. Ginger can acts as an insulin sensitizer and have hypolipidemic and antioxidant effects. Several animal studies have demonstrated the potential of ginger's hepatoprotective effects on the NAFLD (Gao, et al., 2012; Lai, et al., 2016). Possible diagnosis of NAFLD is usually made with a positive imaging study (usually ultrasound) in the absence of persistently elevated serum aminotransferases, a history of alcohol use and other congenital or chronic liver diseases. At the same time, 4 to 5% of patients with other chronic liver diseases might have NASH, and significant autoantibody titers can be found in 20% of NAFLD patients (Brunt, et al., 2003; Cotler, et al., 2004; Vuppalanchi, et al., 2012). Liver biopsy is necessary to accurately classify liver disease and exclude other liver diseases encountered.

1.2.4 Hepatocellular Carcinoma

Several rat studies have mentioned that ginger supplementation can suppress liver carcinogenesis by scavenging free radical formation and reducing lipid peroxidation (Yusof, et al., 2008; Habib, et al., 2008). Ginger may act as an anti-inflammatory and anticancer agent by inactivating NF-κB through suppression of proinflammatory tumor necrosis factor-α (Habib, et al., 2008).

1.3 Ginger as Antioxidant

1.3.1 Health Relevant Active Compounds

Antioxidants are found in the nutraceuticals, referring to foods that have health benefits beyond the nutritional requirements and are used to treat or prevent certain diseases. Nutraceuticals are plants, seeds, vegetables and roots, as they internally produce their own antioxidants against oxidative stress, which offer a natural source of antioxidants. Flavonoids, carotenoids, benzoic acids, cinnamic acids, folic acid, ascorbic acid, tocopherols, and tocotrienols are some of the natural antioxidants produced by the botanicals mentioned above for their oxidative protection (Ghasemzadeh, et al., 2010). Oxidative damage to living tissues induces an inflammatory response and can also lead to an increased risk of chronic diseases such as cancer, coronary atherosclerosis, and other age-related degenerative diseases (Astley, 2003; Stoilova, et al., 2007). It has also been proven that dietary antioxidants are found in nutraceuticals and prevent or eliminate the botanical accumulation of harmful oxidative products and are beneficial to human health.

1.4 Importance of the Liver for Our Body

The liver is one of the most important organs in the body. Any dysfunction that may occur in the liver affects all systems in the body. Chemicals, drugs, alcohol, accidents, liver tumours, viral induced liver disease, liver injury and surgery arising directly from disorders of effective body may cause liver damage (Uyanoğlu, 2006). The liver is more advantageous than other organs in the body with its ability to repair itself. When the liver is damaged due to different reasons, proliferation and replication can start to complete livers functional mass (Fausto, 2000; Başoğlu, et al., 2000). Medically, there are many drug applications are used to improve the damaged liver more quickly. However, as with many chemical drug treatments, side effects are unavoidable. Therefore, the use of natural substances has become widespread in recent years. The majority of these substances are plants in nature and are normally used by the public. For example Silymarin it is obtained from plant *(Silybum marianum* L.), which is used for liver health and has scientifically proven benefits also silymarin has positive effects on liver regeneration (Uyanoğlu, 2006).

1.5 Histology of the Liver

The liver is the body's largest organ at the same time the largest gland after the skin. It is considered as an auxiliary gland belonging to the digestive system. Livers weight is about 1.5 kg (Junqueira, et al., 1998). As bile drains into the duodenum through the bile ducts, exocrine is an endocrine gland because it synthesizes proteins such as albumin, fibrinogen, globulins, prothrombin, lipoproteins, and glucose and directly releases these substances into the blood (Junqueıra, et al., 1998; Karaöz, 2002; Paker, 1986). The liver is covered with the peritoneum all around, except for the parts that contact the diaphragm and the abdominal wall on its back. This serous membrane, which covers the liver from the outside, is called the visceral peritoneum. This membrane consists of mesothelium, a type of single-layer flat epithelium, and a thin connective tissue below. There is a connective tissue rich in elastic fibers that are located under the peritoneum and surround the organ completely externally called Glisson capsule. In the Hilus area, Glisson capsule enters inward and divides the organ into lobes and lobules. Eventually, the liver is with these connective tissue compartments, it is divided into "liver lobules" of 1 mm in diameter and 1-2 mm in length, reaching approximately 1 million. In histological sections, lobules are observed as irregular hexagons arranged side by side like honeycomb. Since the connective

tissue that divides the liver consisting of 4 lobes into lobules is developed very little in humans, the lobule borders cannot be easily distinguished. However, in animals such as camels, pigs and polar bears, these compartments are very well developed and the lobules can be easily separated from each other in the form of irregular hexagons. Only reticulum fibers are found in the connective tissue within the lobule. Reticulum fibers, located between liver cells and sinusoids, serve as a carrier of the liver parenchyma. The connective tissue increases in the sections where the lobules meet each other and creates triangle shaped areas in cross sections. These connective tissue areas containing arteries, veins and bile ducts are called portal Glisson triangle (Erbengi, 1994; Erkoçak, 1982; Gartner, et al., 1997; Karaöz, 2002; Paker, 1986).

1.6 Liver Lobules

The parenchyma of the liver consists of hepatocytes. How the hepatocytes are arranged in the parenchyma and its anatomical and functional validity are still controversial. There are three important models accepted for the regulation of the liver parenchyma (Figure 3a and Figure 3b).

1.6.1 Classic Liver Lobule

The classical liver lobule consists of the vena centralis in the middle, liver cell cords extending radially from the vena centralis to the periphery, and sinusoids located between these cords. Except for the lobules under the full capsule, most of them have apex directed towards the hilus. The number of lobules is about 1 million. In cross sections the lobule is chosen in the form of a hexagon. At each corner of the lobule, there is a vena centralis in the middle of the Glisson triangle. Radially anastomosed with each other around the vena centralis, as branching hepatocytes form an epithelial network (reticulum) the term reticular gland is also used for the liver. Liver cell cords with a single cell thickness are called Remark cords or liver cell cords. Sinusoid type vessels located between liver cell cords show a reticular arrangement with bile ducts and reticulum fibers. This hexagonal structure unit formed by the liver cells around the vena centralis is called the classical liver lobule. Although this model facilitates the microscopic examination of the liver, it cannot

fully explain the liver functions (Paker, 1986; Erbengi, 1994; Gartner, et al., 1997; Karaöz, 2002).

1.6.2 Portal lobule

This model is called Mall's "portal lobule". Especially the fact that some diseases cause degeneration in specific regions of the liver parenchyma has led many researchers to design different structural models. Adjacent liver cells delivering bile to a bile duct within the portal space is also called the portal lobule. The portal lobule is demarcated by combining the vena centralis of 3 classical liver lobules. Although the portal lobule model is a more functional model compared to the classical liver lobule, however, it still cannot explain some pathologies (Paker, 1986; Erbengi, 1994; Karaöz, 2002).

1.6.3 Portal Acinus (Hepatic Acinus)

This model was developed by Rappaport and his colleagues. It is more functional and more accepted model compared to the other two models. From the same interlobular vein within two adjacent classical lobules, cell groups that are bleeding are defined as hepatic acinus. The interlobular vein that runs between the lobules is distributed into two adjacent lobules. Its boundaries are drawn by joining 2 vena centralis and 2 portal spaces. It is in the form of a diamond in cross sections. In multi-functional organs, there are cytological differences between cells with different functions. In the liver, hepatocytes can perform many different missions together. However, considering the feature in the blood supply of hepatocytes, functionally it is possible to separate hepatocytes into 3 zones (Paker, 1986; Erbengi, 1994; Karaöz, 2002).

1.6.3.1 Peripheral Zone (Zone I)

As the blood vessels run from the periphery of the lobule towards the center, Peripheral cells that encounter the blood richest in glycogen, oxygen and other substances show continuous activity. These cells are the first to be affected by harmful substances in the blood. Glycogen is mostly stored in these cells and less frequently in the inner zones. However, when it comes to supply glycogen back into the blood in case of hunger or fasting, central cells first discharge their glycogen. Until glycogen is emptied in cells in central zone no glycogen is delivered from cells in other zones (Aksoy, 2007). Also zone I more active in gluconeogenesis and contains more alkaline phosphatase and transaminases than other sites (Aksoy, 2007).

1.6.3.2 Intermediate Zone (Zone II)

It is a zone with varying activity between the peripheral and central zones.

1.6.3.3 Central Zone (Zone III)

They are resting cells that form a narrow region around the vena centralis. The organelles of cells are less developed than cells in other zones. Cells in this area are particularly rich in the flat endoplasmic reticulum. Pathological and physiological fat accumulation in the liver starts in the cells in the central zone. In some cases such as diet deficiency, fat storage is more in the peripheral zone. In the central zone, drug metabolizing enzymes are at the highest concentration. This area is the most exposed to viral, toxic, and anoxic liver damage (Paker, 1986; Karaöz, 2002).

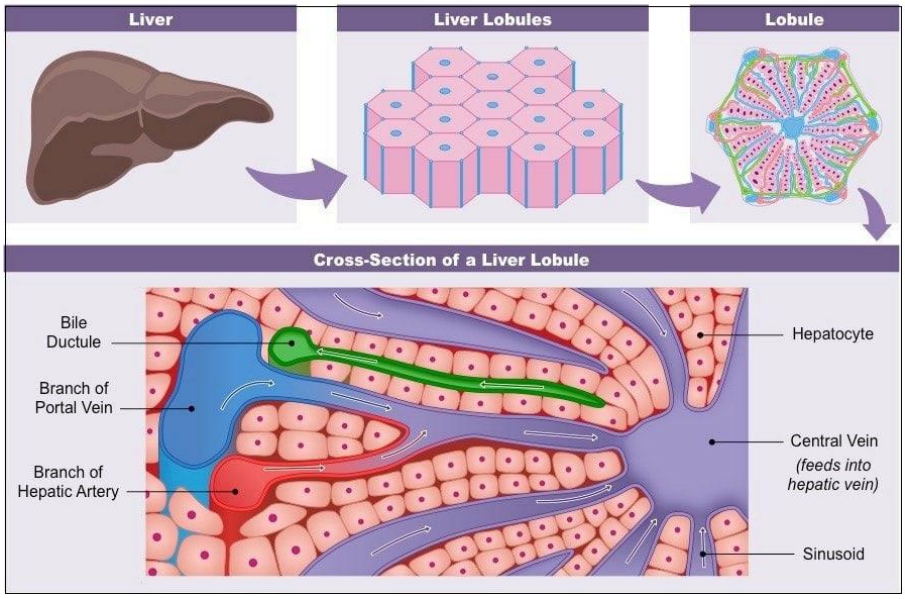
1.6.4 Histological Features of Hepatocytes

Hepatocytes are polyhedral (multi-surface) parenchymal cells of about 20-30μm in diameter, arranged in irregular layers between liver sinusoids. They make up 80% of the liver cell population (Erbengi, 1994). They form cell cords that are one or more cells thick in the central vein radially extending toward the lobules periphery (Azaz, et al., 2004;

Aşıcıoğlu, 2005). There are also sinusoidal capillaries in the spaces between the cell cords consisting of hepatocytes (Erbengi, 1994). Usually they have a single nucleus located in the center, but two nucleus and multinucleated cells are also present, and about 20% of the cells are two nuclei. Often they have one or more nuclei involved while the production of ribosomal RNA (Azaz, 2004). In Hematoxylin and Eosin (H&E) stained parts, because of the presence of multiple mitochondria and some smooth endoplasmic reticulum the cytoplasm of the hepatocyte is eosinophilic (Editorial, 1999). There are three functional surfaces of liver epithelial cells they are, sinusoidal, canalicular and intercellular. On the side of the hepatocytes facing the sinusoids, there are numerous microvilli of irregular shapes and sizes. These structures increase the secretion and absorption area of the cell about six times (Erkoçak, 1982; Aşıcıoğlu, 2005). The space between the hepatocyte and the endothelial cells lining the sinusoids is called the Disse space (Aşıcıoğlu, 2005). Blood in sinusoids passes easily through the endothelial wall and contacts the hepatocyte surface. At the same time, molecules synthesized in the hepatocyte (e.g. lipoproteins, albumin, fibrinogen, prothrombin and coagulation factors V, VII, IX) passes through the Disse into the blood in the sinusoids (endocrine feature of the liver) (Editorial, 1999; Fickert, et al., 2000). There are hepatic stellate cells, also called (Ito cells), located in the Disse range. These cells are of mesenchymal origin, contain fat and have the capacity to store vitamin A in lipid droplets as retinyl esters (Erbengi, 1994). In pathological conditions, Ito cells transform into collagen producing cells. In addition to the synthesis and release of type I collagen, they secrete laminin, proteoglycans and growth factors (Fickert, et al., 2000). They are weak in terms of organelles. Extensions establish relations with neighboring hepatocytes (Aşıcıoğlu, 2005). The face forming the bile canaliculus makes the walls of the part where bile is first secreted (Aşıcıoğlu, 2005). Mutually adjacent membranes of liver cells with bile canaliculi curl occurs. There are short microvilli on this face and the size of the microvilli changes depending on whether bile is secreted or not. When there is no secretion, the microvilli increase in size and close the lumen (Erkoçak, 1982). On the face, which is in close relation with neighbor cell, especially in the regions just below and above the bile canaliculus, there are zonula occludens type tight junction units between the cells. Thus, prevents bile leakage out of this canaliculus. Also coordination of physiological activities of cells is important in the space junctions from intercellular communication sites, are also seen in hepatocytes (Erkoçak, 1982; Erbengi, 1994; Aşıcıoğlu, 2005).

Figure 3a

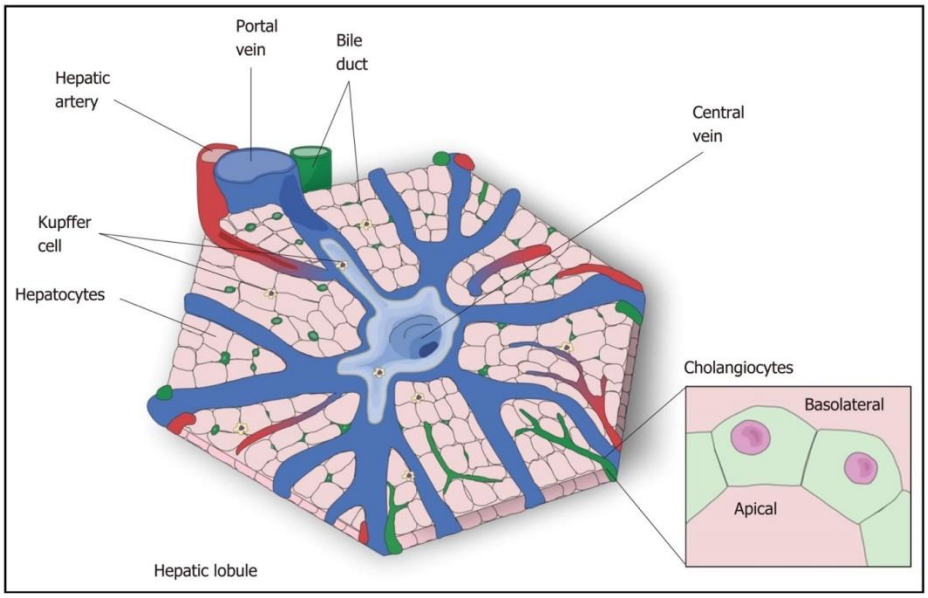
Liver Lobule Structures



(de Oliveira Lemos, et al., 2019)

Figure 3b

Liver Lobule Structures



(de Oliveira Lemos, et al., 2019)

1.6.5 Cytological Features of Hepatocytes

1.6.5.1 Nucleus

Hepatocytes have one or two round nucleus containing one or two typical nucleolus. Some of the nucleus is polyploidy (Editorial, 1999). Mitosis is rare in the adult liver, however, abundant mitosis is encountered during the repair period after damage (Erkoçak, 1982).

1.6.5.2 Mitochondria

There are approximately 2000 mitochondria in each cell. The crystals of spherical or oval shaped mitochondria are numerous. Being rich in mitochondria indicates the high metabolic activity of the cell (Erkoçak, 1982).

1.6.5.3 Endoplasmic Reticulum

Hepatocyte includes the Rough Endoplasmic Reticulum (RER), involved in the synthesis of plasma proteins, also with Smooth Endoplasmic Reticulum (SER), it is associated with glycogen, lipid synthesis, detoxification mechanisms (Fickert, et al., 2000). As a result of the metabolic action of drugs, toxins or metabolic stimuli, the most dominant organelle in the hepatocyte cytoplasm becomes SER. SER hypertrophy develops after the administration of phenobarbital, ethanol, anabolic steroids and progesterone and some cancer drugs, and the enzyme activities related to the binding of these drugs increase. In the hepatocyte, the RER forms scattered clusters within the cytoplasm, these are called basophilic bodies. The excess glucose from the blood is taken by hepatocytes and stored in the cytoplasm as glycogen. In electron microscopic examination, the polysaccharides that called glycogen, is seen as coarse and electron-dense granules collected in RER clusters. Best Carmine and Periodic Acid Schiff (PAS) are suitable colours (dyes) to see the glycogen (Erbengi, 1994).

1.6.5.4 Golgi Complex

It is numerous and located close to the bile canaliculi (Erkoçak, 1982). Their number reaches 50 in each cell. Forming lysosomes and the secreting plasma proteins (albumin), glycoproteins (transferrin) and lipoproteins (Very Low Density Lipoproteins - VLDL) are the functions of this organelle (Editorial, 1999).

1.6.5.5 Lysosome

Lysosomes contain partially digested cytoplasmic organelles called pigment granules (lipofuscin), and various quantities of myelin shapes (Aşıcıoğlu, 2005). Hepatocyte lysosomes also store iron, which is the degradation product of ferritin, in the form of soluble ferritin and insoluble hemosiderin (Fickert, et al., 2000).

1.6.5.6 Lipid Droplets

The presence of different ratios of lipid glycogen and SER in liver cells indicates that the function of hepatocytes is versatile. It is possible to show lipid droplets with Sudan dyeing method (Erkoçak, 1982; Erbengi, 1994).

1.6.5.7 Sinusoids

Around the portal space, liver cells are located in the form of a layer (limiting plate) that is one cell thick against periportal connective tissue (Aksoy, 2007). The branches of the vena porta and the arteria hepatic in the portal space discharge their blood into the vascular system called sinusoid, which is irregularly shaped, special, and larger than the capillaries that present everywhere which located between the liver cell cords. Sinusoids contain both arterial and vein blood. The vena centralis wall contains many holes due to the opening of many sinusoids and the surrounding liver cells form a discontinuous wall (Aksoy, 2007). There are 3 types of cells in the sinusoid wall they are, endothelial cells, Kupffer cells, Ito cells (Paker, 1986; Gartner, et al., 1997; Karaöz, 2002).

1.6.5.8 Endothelial Cell

A basement membrane of very thin reticulum fibres surrounds the endothelial cells. The basement membrane is intermittent in some regions and not found at all in some regions. Endothelial cells are spaced partly or separately. They have flat and dark stained nucleus. Small micropinocytic vesicles can be found in their cytoplasm (Paker, 1986; Karaöz, 2002).

1.6.5.9 Kupffer's Phagocytic Stellate Cell

Between the endothelial cells lining the sinusoids or on the side facing the lumen, another cell type is encountered. These cells, known as Kupffer cells, are members of the mononuclear phagocytic system that is widely distributed in the organism. They are of bone marrow origin and are also known as hepatic macrophages. Their main function is to eliminate circulating harmful agents and old erythrocytes that end life cycle. In addition, they secrete various cytokines that have immunomodulatory functions. These cells are larger than endothelial cells. Their nucleus is oval and large, and their nucleolus is very different. The pale staining and shape of the nucleus allows it to be easily distinguished from the endothelial cell. The irregular cytoplasmic extensions of these cells, adjacent to the endothelial cells facing the lumen, acquire the form of a star. Kupffer cells have neither cytoplasmic connection with each other nor with endothelial cells. Since they are phagocytic cells, their cytoplasm contains abundant lysosomes and phagocytic products (such as iron, pigment released from erythrocyte breakdown). The peroxidase reaction is positive, so it can be easily separated from the peroxidase negative endothelial cells. Again, as a result of injections containing dye particles, it is easy to identify these cells with phagocytic properties. The cells are rich in term of organelles (Aksoy, 2007). Like other macrophages, they are derived from the bone marrow, but experimentally they have been shown to reproduce by mitosis after stimulation. In some cases, the Kupffer cell leaves the sinusoid wall and enters the circulation through the vena hepatica. They also can store vitamin A (Paker, 1986; Karaöz, 2002).

1.6.5.10 ITO Cells

In the Disse space, there are fat storage cells, known as Ito cells. These cells contain lipid residues rich in vitamin A. These cells perform various functions in healthy liver like uptake, storage and secretion of retinoid, secretion and synthesis of some extracellular matrix proteins and proteoglycans, secretion of growth factors and cytokines, and also regulation of sinusoid lumen diameter in response to many regulatory substances such as thromboxane A2 and prostaglandins. Ito cells are cells with long cytoplasmic extensions under the endothelium and in sinusoids, providing the connection between hepatocytes. They also connect with each other and with nerve endings. Electron microscopic view of Ito cells, it is characterized by nucleus compacted fat droplets, partially developed RER, reduction in cytoskeleton structures, few mitochondria. It is difficult to identify Ito cells in sections stained with Hematoxylin and Eosin. It is possible to observe these cells by using histological stains such as basic fuchsin, toluidine blue, Oil red O as special staining. Numerous Ito cells were observed in centrolobular areas in human liver tissue sections with silvering technique. Observation of Ito cells is dependent on vitamin A staining. By immunohistochemistry, Ito cells can be defined as antibodies to cells forming the cytoskeleton. Desmin is a good identifier for Ito cells in rats. As in many cells of mesenchymal origin, vimentin is identified in rat stellate and human cells. Rat Ito cells contain Glial Fibrillary Acidic Protein (GFAP) major component of intermediate filaments in astrocytes. Ito cells also contain Neural Cell Adhesion Molecule (N-CAM). The Ito cells are also called "livers specific pericytes". Because with the markers, they shows similarities to astro glial and neural derived cells. Examples of intermediate filament expression are striking. Besides normal functions of Ito cells, they have different functions in acute and chronic liver disease, vitamin A intoxication and liver tumours. In chronic liver disease, fibrosis and cirrhosis, many cells like myofibroblast are observed by Ito cells. Ito cells have an important role in liver fibrogenesis. The number of Ito cells increases and the amount of intracellular fat droplets also increase in the early stage of chronic vitamin A intoxication (Paker, 1986; Gartner, et al., 1997; Karaöz, 2002).

1.6.5.11 Disse Space - Perisinusoidal Space - Subendothelial Space

Between the liver cells and sinusoid wall a narrow space exists. This space is called the Disse space, Perisunusoidal space, Subendothelial space. This range can be selected under electron microscopy between the liver cell and the endothelium, where the microvilli of hepatocytes are located. The blood in the sinusoids passes easily through the endothelial cells and easily contacts the hepatocyte surface. Although the fluid here is in plasma quality, although, Disse is not the lymphatic space, but the interstitial space. However, Disse space plays an important role in production of large amounts of lymph in liver (Parker, 1986). Disse space continues with Mall space at the periphery of the lobule. The Mall space is located around the bile ducts and vessels in the portal interval. Liver lymph vessels begin as blind ends from this space. Within the disse space there are a small number of Perisunusoidal cells that can derive from both the endothelial cell and the Kupffer cell. Perisinusoidal cells can synthesize reticular and fibrogen fibril, as typical fibroblasts are absent in the Disse space. According to some researchers, endothelial cells are the place of growth of fibrils within the Disse space. Perisinusoidal cells contain oil droplets in the cytoplasm. Physiological significances are not known well. They are not phagocytic. In the fetal liver, these cells likely act as stem cells that provide hemopoiesis. Perisinusoidal cells are mostly found in the intermediate and peripheral zones, but few in the central zone (Parker, 1986).

1.7 Liver Histophysiology

1.7.1 Carbohydrate Metabolism

The liver, along with the anterior lobe of the pituitary and adrenal cortex, plays a role in all stages of carbohydrate metabolism. The liver converts glucose, fructose and galactose into glycogen and stores it (glycogenesis). It also converts excess carbohydrates into fat for storage. When food is not taken or when blood sugar is decreased, the liver breaks down glycogen and keeps blood glucose normal to provide energy needs (glycogenolysis). Hepatocytes convert glycerol fragments of lipids and amino acids into glucose through an enzymatic pathway called gluconeogenesis. In the state of satiety, the liver converts glycogen into glucose and sends it to cells that do not have their own synthesis stores such as red blood cells and central nervous system cells (Wallach, 2000; Karaöz, 2002).

1.7.2 Protein Metabolism

Liver also plays an important role in the protein metabolism. The liver synthesizes many plasma proteins. These, transport and binding proteins (albumin, transferrin, ceruloplasmin, haptoglobulin), protease inhibitors (antithrombin III, alpha 1- antitrypsin), hemostasis proteins (prothrombin, fibrinogen) and tissue inflammation proteins. Remaining gamma globulins are antibodies and are also made in plasma cells mainly in lymphatic tissue. The rate of production of plasma proteins per day in the liver can be a maximum of 15-50 grams. Hence, even if half of the plasma proteins lost in the body, this amount could be replaced in a week or two. Decrease in plasma proteins accelerates mitosis in liver cells and leads to liver enlargement. Most albumins are produced by the liver and indicate liver function. Ig plasma levels also change in liver diseases. The liver is also responsible for nitrogen metabolism. Converts amino acids to urea with the cycle of ammonia and krebs. In severe liver damage, urea nitrogen decreases in the blood, ammonia and amino acids increase. The deamination of amino acids is another function of the liver. Amino acids must be deaminated before they can be converted to carbohydrates or fats or used for energy. It covers a very small percentage of that in the liver, particularly the kidneys. Synthesising amino acids then forming important chemical compounds from amino acids is one of the major functions of the liver. For example, all non-essential amino acids could be synthesized in liver. The liver removes ammonia from body fluids through the formation of urea. The large amount of ammonia, which is results from deamination processes, is continuously made by bacteria in the intestines and absorbed into the blood. Therefore, in the absence of urea production in the liver, the ammonia concentration in plasma rises rapidly and death is seen with hepatic coma. Indeed, even when the liver blood flow is much reduced, infrequently, large amounts of ammonia accumulates in the blood at the shunts between vena cava and portal vein, leading to a very toxic situation (Guyton, 2001, Karaöz, 2002).

1.7.3 Lipid Metabolism

Although fat metabolism is partially carried out in all cells in the body, some processes of this metabolism are carried out mostly in liver. The specific functions of the liver in fat metabolism are as follows, rapid oxidation of fatty acids to provide energy for bodily functions, the formation of most lipoproteins, the synthesis of large amounts of phospholipids and cholesterol, carbohydrate conversion and protein to fat. In order to get energy, neutral fats are first broken into glycerol and fatty acids. Fatty acids are then split into two-carbon acetyl radicals by beta-oxidation. These form Acetyl Coenzyme A (Acetyl-CoA). Acetyl-CoA enters the citric acid cycle, oxidizes then provides a large amount of energy. Although beta oxidation is done in all cells in the body, this process is particularly rapid in liver cells. Acetoacetic acid, which formed by combination of the two molecules of Acetyl-CoA, dissolves very easily and passes from liver cells to extracellular fluids and it is carried to the whole body and absorbed by tissues. Tissues also oxidize acetoacetic acid in the normal way by converting it back to Acetyl-CoA. Therefore, it is natural or normal that the liver is largely responsible for fat metabolism. Approximately 80 percent of the cholesterol synthesized in liver will turn to bile salts then secreted into bile. The remainder is carried to all tissue cells of the body as lipoproteins through the blood. In liver phospholipids are synthesized in the same way and they are mostly transported in lipoproteins. Fat synthesis from proteins and carbohydrates in the body takes place largely in liver. After its synthesis in liver, fat will be transported to the fat tissue in lipoproteins then stored (Guyton, 2001).

1.7.4 Storage of Vitamins

The liver has the ability to store vitamins. It has long been known that the liver is a great source of vitamins for the patients treatment. Vitamin A is the only vitamin that is stored in large amounts in the liver. Normal amounts of vitamin D and vitamin B_{12} are also stored at the same time. Vitamin A can be stored in high amounts for up to ten months to prevent liver vitamin A deficiency, also stores enough vitamin D to avoid vitamin D deficiency for three to four months, and also stores vitamin B_{12} to avoid deficiency of B_{12} for at least a year or more (Guyton, 2001).

1.7.5 Liver and the Blood Coagulation Relationship

Most of the substances used in the coagulation process in the blood are synthesized in the liver. These substances are accelerator globulin, fibrinogen, factor VII and prothrombin. Metabolic events in formation of prothrombin and VII, IX, X factors in liver will require vitamin K. In the absence of vitamin K, coagulation is almost completely eliminated since the concentration of these substances is very low (Guyton, 2001; Karaöz, 2002).

1.7.6 Iron Storage

Apart from the iron found in hemoglobin in the blood in the body, most of the iron is stored in the liver as ferritin. Apoferritin, a protein that can combine with iron in high or low amounts, liver cells are rich in apoferritin. Thus, when the amount of iron increases in body fluids, ferritin is formed by combining with apoferritin and is stored for use in another place when necessary. When the amount of iron in the circulating body fluids decreases, ferritin will release iron. Thus, in liver ferritin and apoferritin systems act as both an iron store and a buffer for iron in the blood (Guyton, 2001; Karaöz, 2002).

1.7.7 Excretion of Medications, Hormones and Other Substances by the Liver

Due to the very active chemical environment in the liver, many drugs such as, sulfonamide, penicillin, ampicillin, erythromycin are excreted by bile after detoxification. Likewise, most steroid hormones like thyroxine, cortisol, estrogen and aldosterone secreted by the endocrine glands. Thus, in liver damage, accumulation of one or more of these hormones in the body fluids often leads to overactivity of the hormonal system. In addition, one of the significant ways of excretion of calcium in the blood is to pass into bile and be removed by feces (Guyton, 2001; Karaöz, 2002).

1.8 Regeneration of the Liver

Liver regeneration feature is quite high. The organ returns to its normal weight shortly after surgical removal of part of the liver or administration of toxic (hepatotoxic) substances (such as carbon tetrachloride, chloroform). When 75% of the liver is removed in rats, it is seen that the tissue lost within a month is replaced. In humans, this feature is a bit more limited. Regeneration is achieved by the mitosis and growth of the remaining unspoiled hepatocytes. In the restored liver, parenchymal cells appear to be larger than normal and binuclear cells proliferate. In addition, new liver cells can be formed by budding and even being different in interlobular bile ducts. The mitosis incidents in the liver are controlled with mitosis inhibitory substances circulating in the blood. In case of tissue damage or partial removal, the amount of mitosis inhibitory substances decreases, as the mitosis pressure is removes, a rapid mitosis is observed in the liver. As the regeneration progresses, the amount of mitosis inhibitory substances increases then mitosis gradually decreases and ends. This system is a self-regulating control mechanism. It has been found that a similar mechanism exists in other tissues apart from the liver. In case of permanent or repeated liver damage, the amount of liver connective tissue increases gradually as the increase of connective tissue with liver cell regeneration increases and the pathological condition called cirrhosis occurs (Paker, 1986; Kumar, et al., 2000). Experimentally, administration of carbon tetrachloride to experimental animals causes cell damage in the central zones of liver lobules. While necrotic hepatocytes are destroyed by autolysis, regeneration begins with the mitosis of cells in the peripheral zone of the lobule. Cellular damage is repaired within 5-6 days. If the destruction of the liver started from the periphery of the lobule (for example, in case of obstruction of the bile duct or administration of certain hepatotoxic substances), mitosis is seen all over the healthy tissue, and also, excessive mitosis in the large and small bile duct epithelium remarkable. The amount of bile ducts increases. These ducts enter through the damaged peripheral zone and connect with the bile canaliculi in the intact area. Thus, bile flow interrupted by the destruction of peripheral cells finds a new way for itself. If the damage continues, the number of ductus increases further. If it does not continue, the liver quickly regains the normal structure and new ducts disappear (Paker, 1986).

CHAPTER II

MATERIALS AND METHODS

2. METHODS

Livers, testes, kidneys and the cardiac muscles of the experimental animals will be removed by careful dissection, washed with cold saline solution and dried between two filter papers then photographed for gross observation. Parts of the organs will be taken and then divided into two parts, 0.5 cm^3 each. One part is going to be fixed in 10% formal saline solution for preparation of paraffin blocks and other part will be cut to a very small pieces and it will then be fixed in the 2.5% phosphate buffered glutaraldehyde for the microscopic study.

2.1 Reagents and Materials

1. Acetone
2. Xylene
3. 95-100 % Ethanol
4. Distilled water
5. The reaction buffer
6. Tissue slides with H&E stains
7. Sample tissues embedded in paraffin
8. Microscope slide
9. Slide coverslips

2.2 Equipment's

1. Plastic staining dish (container)
2. Slide Baskets
3. Forceps
4. Wipes
5. Bright field microscope

6. Parafilm
7. Slide Scanner
8. Incubator
9. Slide Cover slipper

2.3 Preparation of the Plant Extract

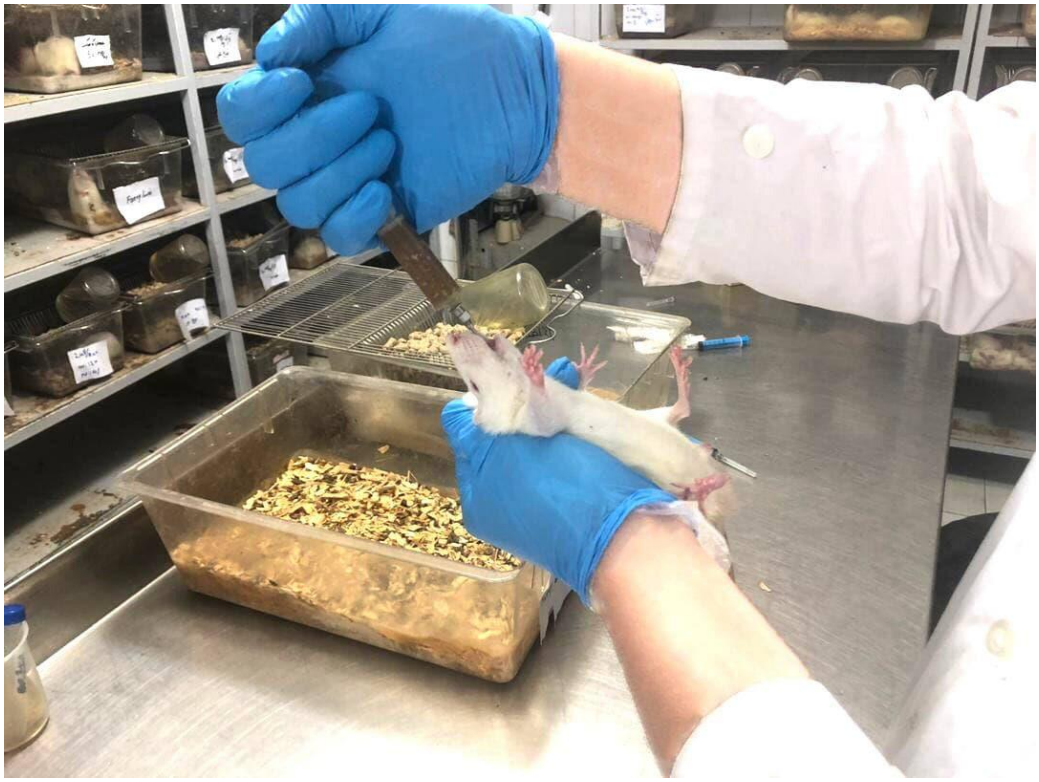
Fresh ginger rhizome purchased from the market in Erbil Government, Iraq. The roots were identified and authenticated by the botanist at Erbil Polytechnic University. Fresh ginger rhizomes (5 kg) were cut into pieces and dried at 50 ° C for three days. The dried material was pulverized with a grinding machine. Dried ginger rhizome powder was extracted with 95% ethanol (4 L) for 48 hours at room temperature. It was then filtered and evaporated on a rotary evaporator to dryness at 50 ° C under vacuum to give the crude ethanolic extract. The ginger paste obtained was collected from the tray with a spatula in a container and measured using an electric weighing scale. 150 g of ginger paste extracted and stored at 4°C until used.

2.4 Experimental Animals

In this study, thirty adult male albino rats aged 3 to 4 months and weighing about 150-180g having the same biological and physiological characteristics were used which was obtained from the experimental animal breeding center of Erbil Polytechnic University (EPU), Erbil, Iraq. The rats were housed in clean stainless and appropriately ventilated cages under similar conditions, and the animals were fed with the composition of the standard laboratory diet. All animals were kept in a temperature controlled room kept at a range of 28-32 °C and humidity 45-50 %, in stainless steel cages, lights were organized as 12 hours light and other 12 hour dark cycle. Animals were acclimatized to their environment 2 weeks before starting the experiment (Guide for the Care and Use of Laboratory Animals, 2011).

Figure 4

Gavage Method



2.4.1 Experimental Design

Following acclimatization, the rats divided to five main groups as follows;

- **Group I** (Control group): Will be included six rats that kept on standard diet for the 5 weeks.
- **Group II** (Fatty liver induced group): Will be included six rats that going to be fed on high-fat diet for 5 weeks.
- **Group III** (Ginger-treated Group): Will be included six rats that are maintained on the standard diet and ginger extract will be given orally by gastric tube at the dose of 200 mg/kg of body weight daily for 5 weeks (Figure 4).
- Protective group: Will be included twelve rats that going to be subdivided into two equal subgroups; six rats each:
- **Subgroup IV:** Rats will be fed on high-fat diet at the same composition and duration as in Group III concomitantly with ginger extract at the same dose and route as in Group II.
- **Subgroup V:** Rats will be maintained on the standard diet and ginger extract will be given for 5 weeks as in Group II then stopped and the rats are being shifted from standard diet to high-fat diet as in Group III for an additional 5 weeks (Figure 5).

2.4.2 Blood Sampling

At the end of the experiments, rats were starved overnight before being sacrificed. To sedate the animal, thick Gamgee with a hole in the middle was placed on the animal's face and a few drops of chloroform were added during the first minute only. From the cardiac puncture blood samples were taken. To get the serum blood was allowed to coagulate at room temperature for 30 minutes and then centrifuged at 4000 rpm for 15 minutes. For biochemical analysis the separating serum was kept frozen at –20°C until used. Liver, testis, kidney and cardiac muscle tissue were immediately removed out and prepared for the histological studies.

Figure 5

Groups

2.4.3 Histological Study

Some liver pieces were immediately fixed in 10% neutral formalin. After fixation, the liver sampled for histological examination was dehydrated and embedded according to standard sample trimming procedures. Livers, testis, kidney and cardiac muscle of the experimental animals were removed by careful dissection, washed with cold saline solution and dried between two filter papers then photographed for gross observation (Figure 6 and Figure 7). Parts of the organs were taken and then divided into two parts, 0.5cm^3 each. One part was fixed in 10% formal saline solution for preparation of paraffin blocks. After fixation organs were sampled for histological examination, dehydrated, then, it was cleaned in xylene and was embedded in the paraffin wax. Samples were cut at 4 µm by a rotary microtome then stained with H&E (Figure 8). Photomicrographs were taken and magnification was 100x and 400x (Bancroft & Gamble, 2008).

2.4.3.1 Immunohistochemical Study of Ki-67

In a graduated series of alcohol solutions deparaffinized sections was hydrated. Sections were incubated at antigen retrieval (the sections boiled for 20 minutes in 10 mmol/L sodium citrate buffer at 98°C), then treated with 3% H_2O_2 to block endogenous peroxidase. Monoclonal antibody (Anti-Ki-67) was applied to the slides and incubated overnight in the refrigerator at 40 ^{0}C in a humid room. Secondary biotinylated antibodies were then applied, followed by incubation with streptavidin peroxidase. After each step the sections were washed three times with Phosphate Buffered Saline (PBS). Sections stained with Diaminobenzidine Chromogen Solution (DAB) and then counterstained with hematoxylin (Horiguchi, et al., 2007). A semi-quantitative estimation of Ki-67 made based on the percentage of positive and staining intensity cells to evaluate the labeling index, from which randomly 5 to 8 sites were selected each sample (an average of 500 hepatocytes). Positive cells counted in sequential high-power fields (400x) and results are expressed as the mean number of positive cells for the limited surface area. Statistical analysis using independent t-test and $P<0.05$ were considered statistically significant.

2.5 PROCEDURES

2.5.1 De-Staining H&E Slides

The De-staining procedures were performed in a fume hood by using manual washing bath which contains the reagent solvents listed in the reagent and materials section.

1. H&E index slides were placed in slide baskets to allow manual rinsing.
2. Slides were hand-soaked in acetone for 10 minutes to remove coverslips.
3. The coverslips were gently removed by using forceps to reduce the tissue damage (longer intervals allows coverslips to slide independently) then coverslips were discarded.
4. To remove any remaining adhesive the slides were rinsed moderately in a xylene bath 3 times, then to remove all sealant the slides were seated in bath.
5. To remove the eosin stain, the slides were rinsed (5-6 times) in 95% EtOH for approximately 30 minutes with 3-minute holding intervals between rinses.
6. Slides were rinsed in distilled water and wipes were tapped to remove the excess.
7. To remove hematoxylin slides were rinsed in the reaction buffer (3 to 4 rinses with two minute holding intervals).
8. The slides were left to dry in the fume hood for 5 minutes.
9. Immunohistochemistry (IHC) antibodies assay detection protocols were run on the instruments or in the manual procedure.
10. The completed slides were washed in water with dish detergent to remove any residual reagent.
11. Coverslips and slides were manually dried with the coverslips using the xylene > acetone > 80% EtOH > 90% EtOH > 100% EtOH > xylene.

Figure 6

Control+200 mg/kg: Liver

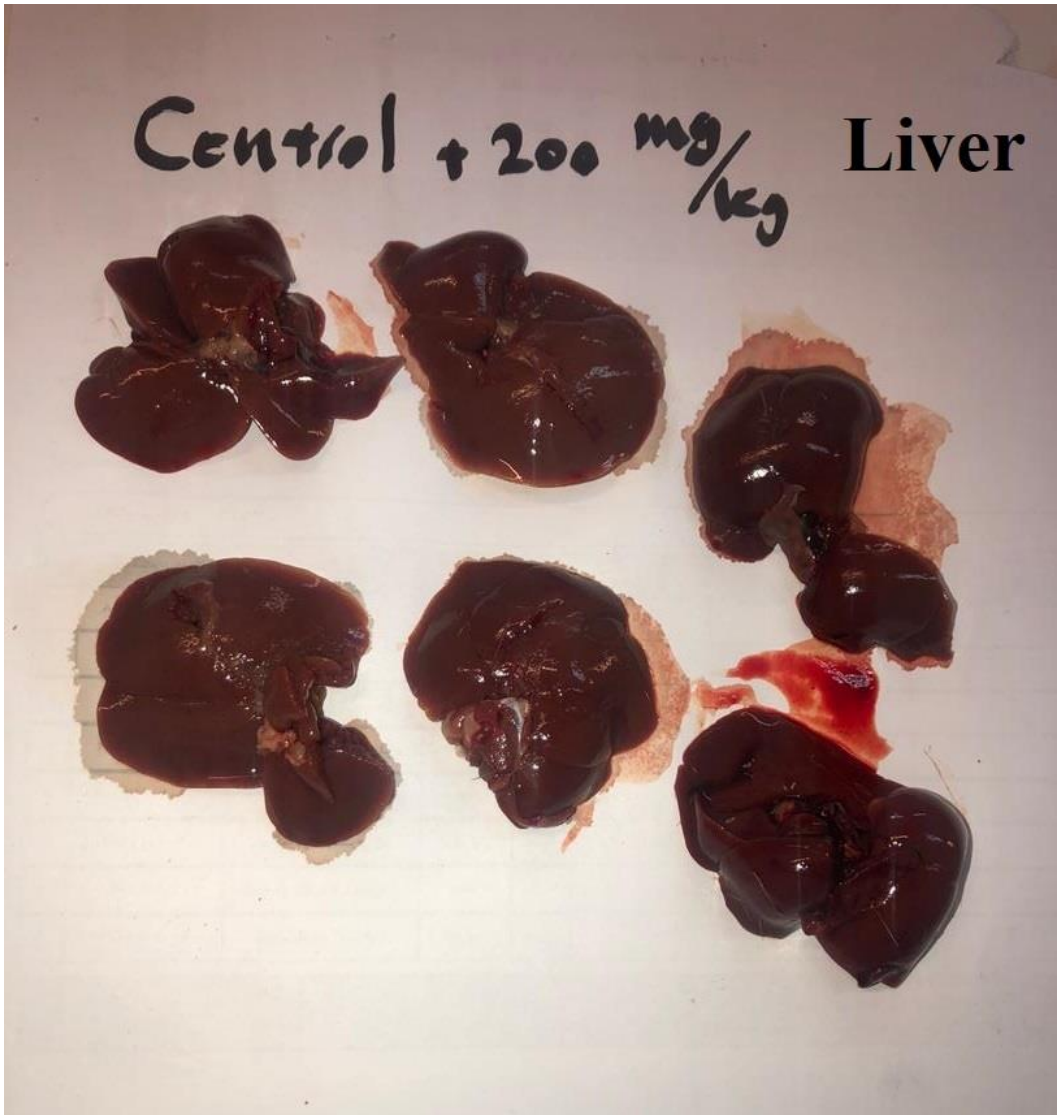


Figure 7

Control + 200 mg/kg: Testes, Kidneys and Hearts

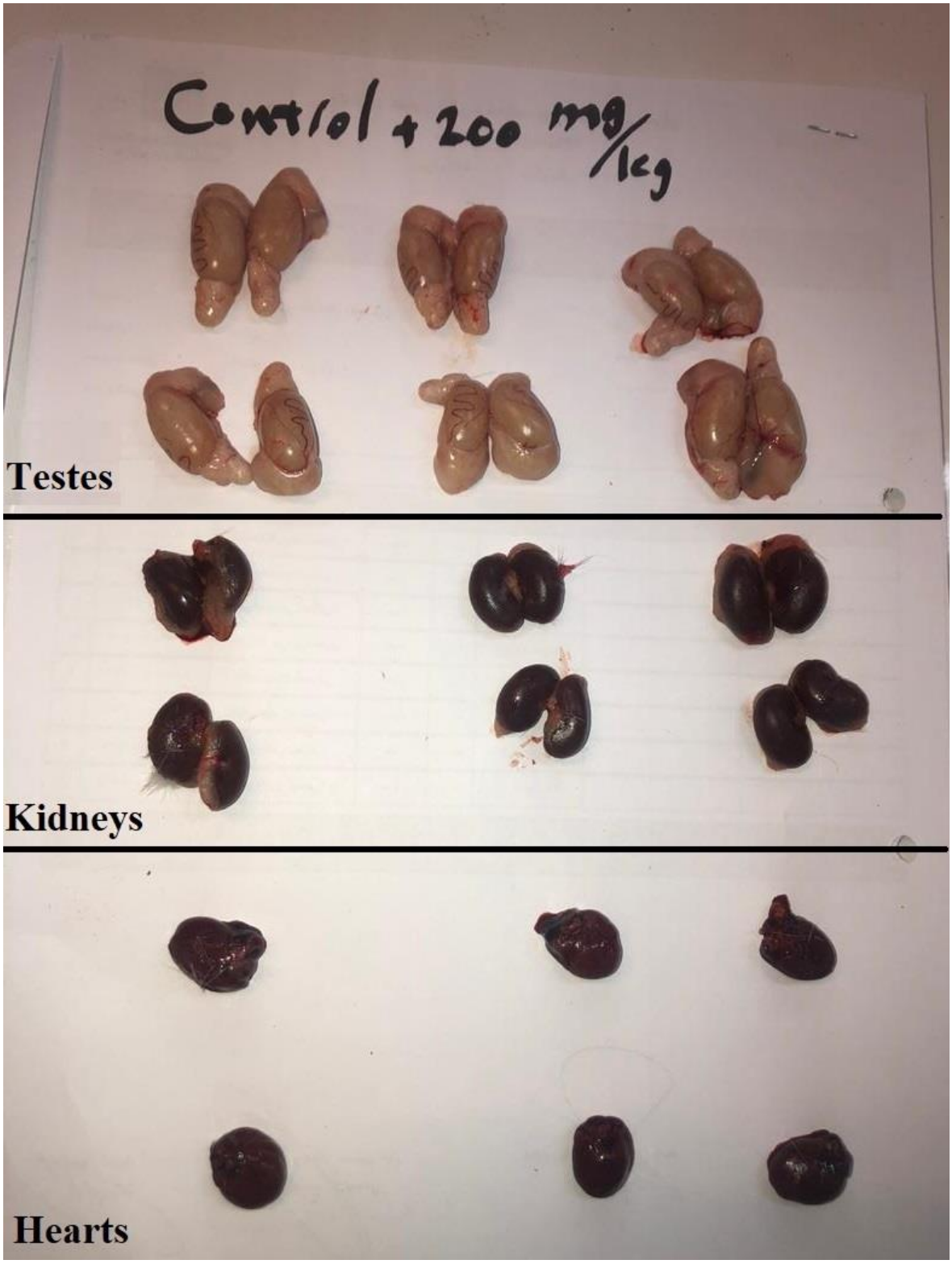
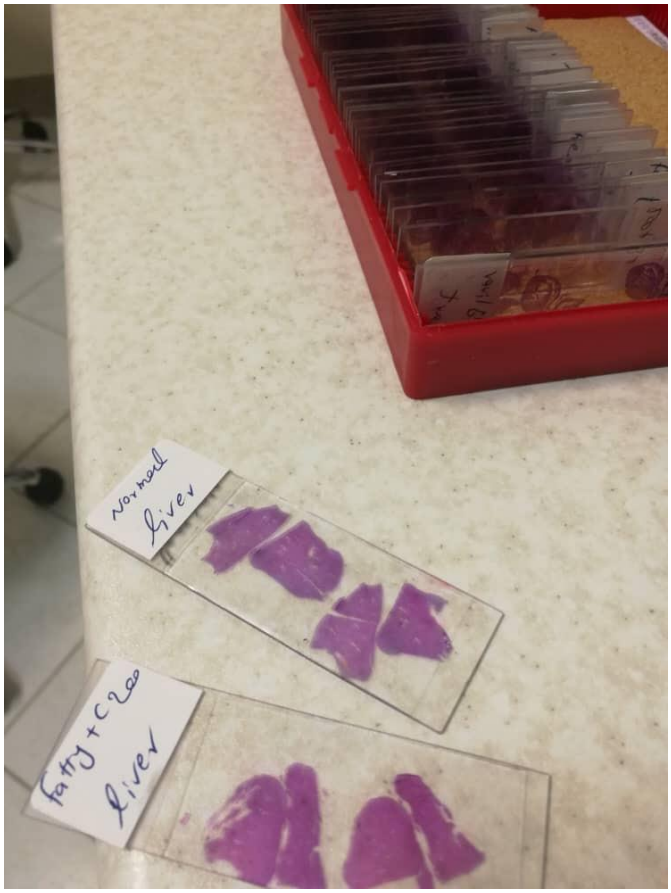


Figure 8

Hematoxylin and Eosin Staining of the Fatty Liver and Normal Liver



2.6 Biochemical Studies

The activities of aminotransferases (ALT and AST), as well as TG, TC, LDL-C and HDL-C and alkaline phosphatise levels estimated colorimetrically by using commercial kits (Randox Laboratories Ltd., County Antrim, UK).

2.7 Statistical Analysis

All groups were expressed as mean ± Standard Deviation (SD) by using SPSS software (SPSS Science, version 11.0.1) statistical analyses were carried out. To compare between the groups post hoc test were used. All information and data's were expressed as means ± SD, $P \leq 0.05$, and 0.001 considered significant and highly significant.

CHAPTER III

3. RESULTS

3.1 Histological Tests

The result of the histological tests was observed that the total cholesterol, triglyceride, SGOT, SGPT and alkaline phosphatase levels of rats in high-fat diet group was significantly ($p<0.05$) increased, with a non-significant ($p<0.05$) increase in HDL and LDL in the fatty liver groups compared to the control groups (Histogram 1.4 A&B). The group that received 200 mg of ginger extract, revealed a statistically significant ($p<0.05$) increase of alkaline phosphatase, SGOT and SGPT level (Histogram 1.3 A&B), with non-significant ($p<0.05$) small effect on cholesterol, triglyceride, HDL and LDL levels was shown in this group as compared to the control groups (Histogram 1.1, 1.2 and 1.4). The results of the group IV and V rats that induced fatty liver with ginger extract, clarified a statistically significant ($p<0.05$) decrease of the alkaline phosphatase, also with a significant ($p<0.05$) decrease of the cholesterol, triglyceride, SGOT and the SGPT levels (Histogram 1.1, 1.2 and 1.3), whereas, non-significant ($p<0.05$) decrease of HDL and LDL levels revealed among the group that induced fatty liver with ginger extract compared with the fatty liver group (Histogram 1.4 A&B). The results of the serum lipid profile seem to support the protective role of this extract in the ginger treated groups. There was no significant difference in kidney, testis and heart tissues when compared to the control group.

3.2 Biochemical Tests

The results of the different estimated fractions and the biochemical analysis of the lipid profiles are summarized in the histograms below. A statistically significant ($p<0.05$) increases of the triglyceride, total cholesterol, SGOT, SGPT and alkaline phosphatase were observed, with non-significant ($p<0.05$) increase in HDL and LDL among fatty liver induced groups as compared with the control groups (Histogram 1.4 A&B). The group that received 200 mg of ginger extract, revealed a statistically significant ($p<0.05$) increase of alkaline phosphatase level. Also, significant ($p<0.05$) increase of the SGOT and SGPT

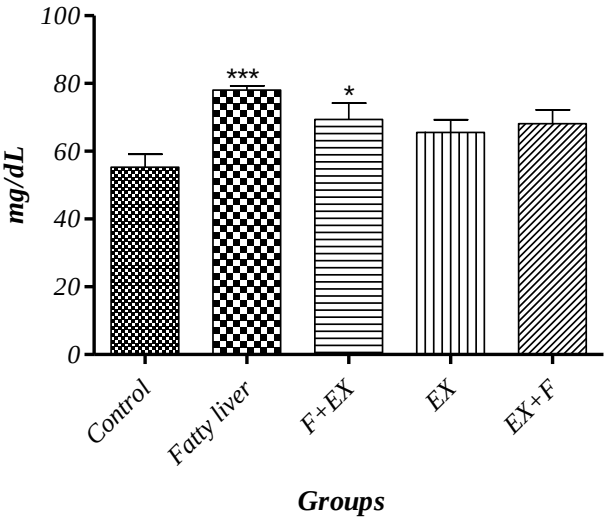
were shown (Histogram 1.3 A&B). However, non-significant ($p<0.05$) small effect on the cholesterol, triglyceride, HDL and LDL levels were shown in this group compared to control groups (Histogram 1.1, 1.2 and 1.4).

The results of the group IV that induced fatty liver plus ginger, revealed a statistically highly significant ($p<0.05$) decrease of the alkaline phosphatase, with a significant ($p<0.05$) decreases of the cholesterol, triglyceride, SGOT and SGPT levels (Histogram 1.1, 1.2 and 1.3), whereas, non-significant ($p<0.05$) decrease of the LDL and HDL levels revealed among the group that induced fatty liver plus ginger extract compared with the fatty liver group (Histogram 1.4 A&B).

The last group (V) that was fatty liver and then treated with the ginger extract showed a statistically high significant ($p<0.05$) decrease of the alkaline phosphatase along with significant ($p<0.05$) decreases of triglyceride, SGPT and SGOT levels, however, this group also showed non-significant ($p<0.05$) decreases of cholesterol, HDL and LDL levels compared to the fatty liver group (Histogram 1.4 A&B).

Histogram 1.1

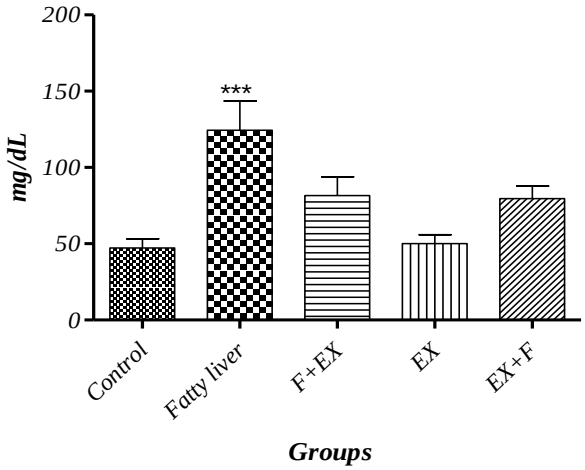
Comparison among different groups as regard means ± standard deviation of cholesterol levels



Control: Control Group; **Fatty Liver:** Fatty Liver Group; **F+EX:** Fatty Liver + Extract Group; **EX:** Extract; **EX+F:** Extract After Fatty

Histogram 1.2

Comparison among different groups as regard means ± standard deviation of triglyceride level

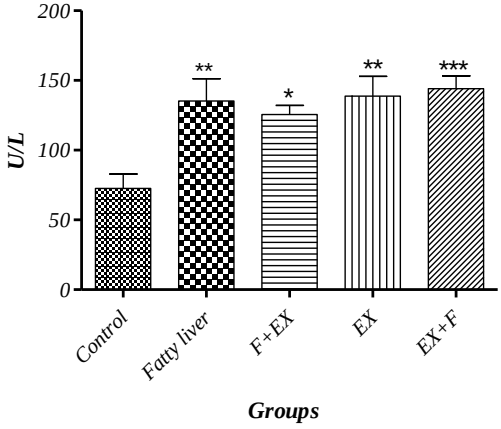


Control: Control Group; **Fatty Liver:** Fatty Liver Group; **F+EX:** Fatty Liver + Extract Group; **EX:** Extract; **EX+F:** Extract After Fatty

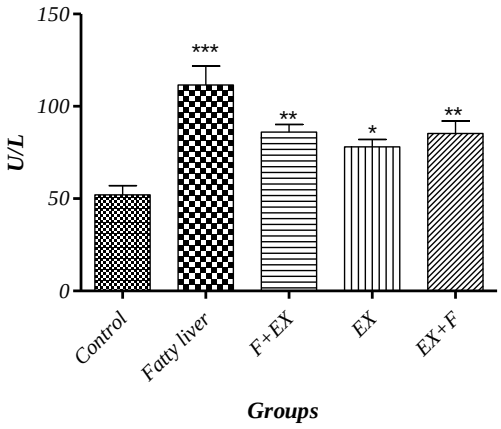
Histogram 1.3

*Comparison among different groups as regard mean ± Standard deviation of **A**. S-GOT and **B**. S-GPT levels*

A.



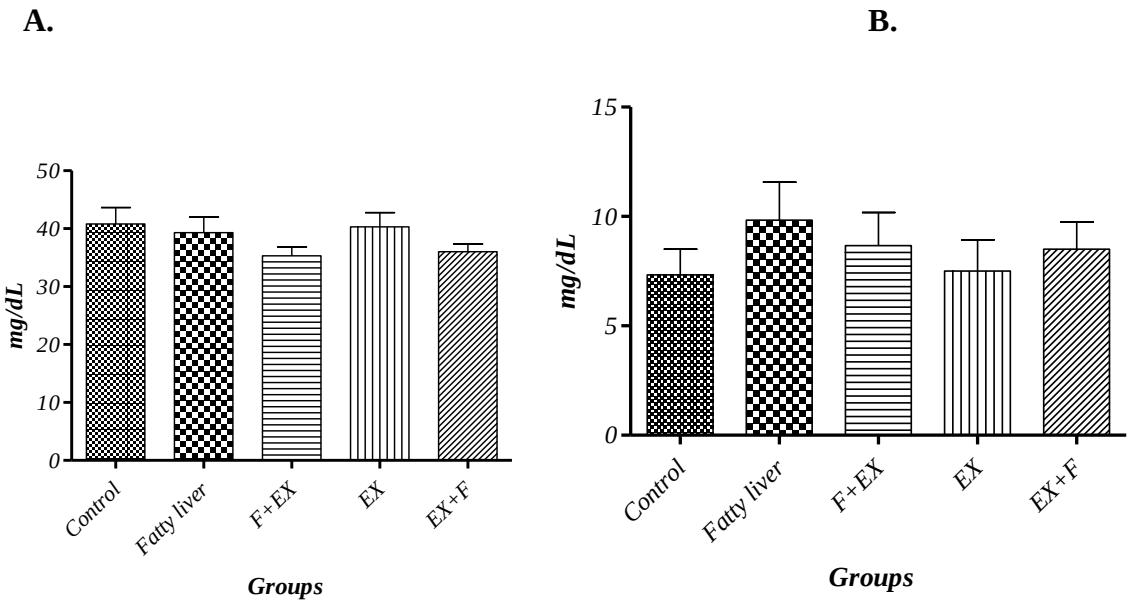
B.



Control: Control Group; **Fatty Liver:** Fatty Liver Group; **F+EX:** Fatty Liver + Extract; **EX:** Extract; **EX+F:** Extract After Fatty

Histogram 1.4

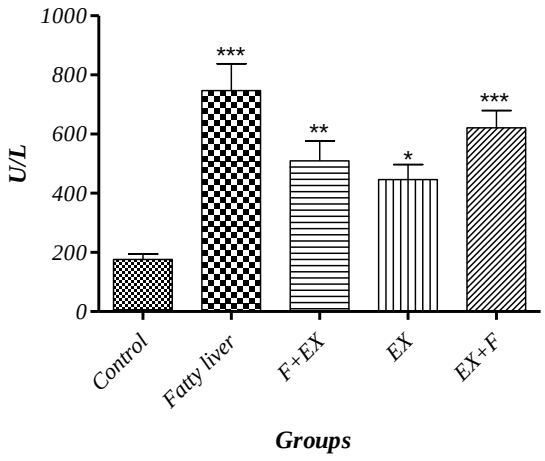
*Comparison among different groups as regard means ± standard deviation of **A**. HDL **B**. LDL levels*



Control: Control Group; **Fatty Liver:** Fatty Liver Group; **F+EX:** Fatty Liver + Extract Group; **EX:** Extract; **EX+F:** Extract After Fatty

Histogram 1.5

Comparison among different groups as regard means ± standard deviation of S-Alkaline phosphatase levels



Control: Control Group; **Fatty Liver:** Fatty Liver Group; **F+EX:** Fatty Liver + Extract Group; **EX:** Extract; **EX+F:** Extract After Fatty

3.3 Light Microscopic Results

3.3.1 Hematoxylin and Eosin Stain Groups

Examination of sections obtained from livers of the control group (Group I) and ginger fed groups (Group III) showed normal histological structure. The liver was formed of classic hepatic lobules which were roughly hexagonal in shape with central veins forming their central axis. At their angles, there were portal areas containing connective tissue stroma and portal triads (Figure 9A: A&C).

Histological examination of liver sections obtained from fatty liver induced group (Group II) revealed several histological changes in the form of disturbed hepatic architecture (Figure 9A: B). Most of the hepatocytes showed variable degrees of cytoplasmic vacuolations, some contained multiple small vacuoles the others appeared ballooned with peripheral nuclei. In addition, some nuclear changes were observed like darkly stained (pyknotic) nuclei, and hepatocytes with nuclear vacuolations (glycogenated nuclei). Hepatocytes with mild necrosis and steatosis (++).

Group (IV&V) exhibited small histopathological changes; few scattered hepatocytes contain steatosis without necrosis.

Figure 9A

The Photomicrographs of the Liver Sections of the Control Group Shows: Normal Liver Architecture, a Classic Hepatic Lobule Containing Central Vein (Star) and Radiating Cords of Hepatocytes with Blood Sinusoids in Between (H&E 100x)

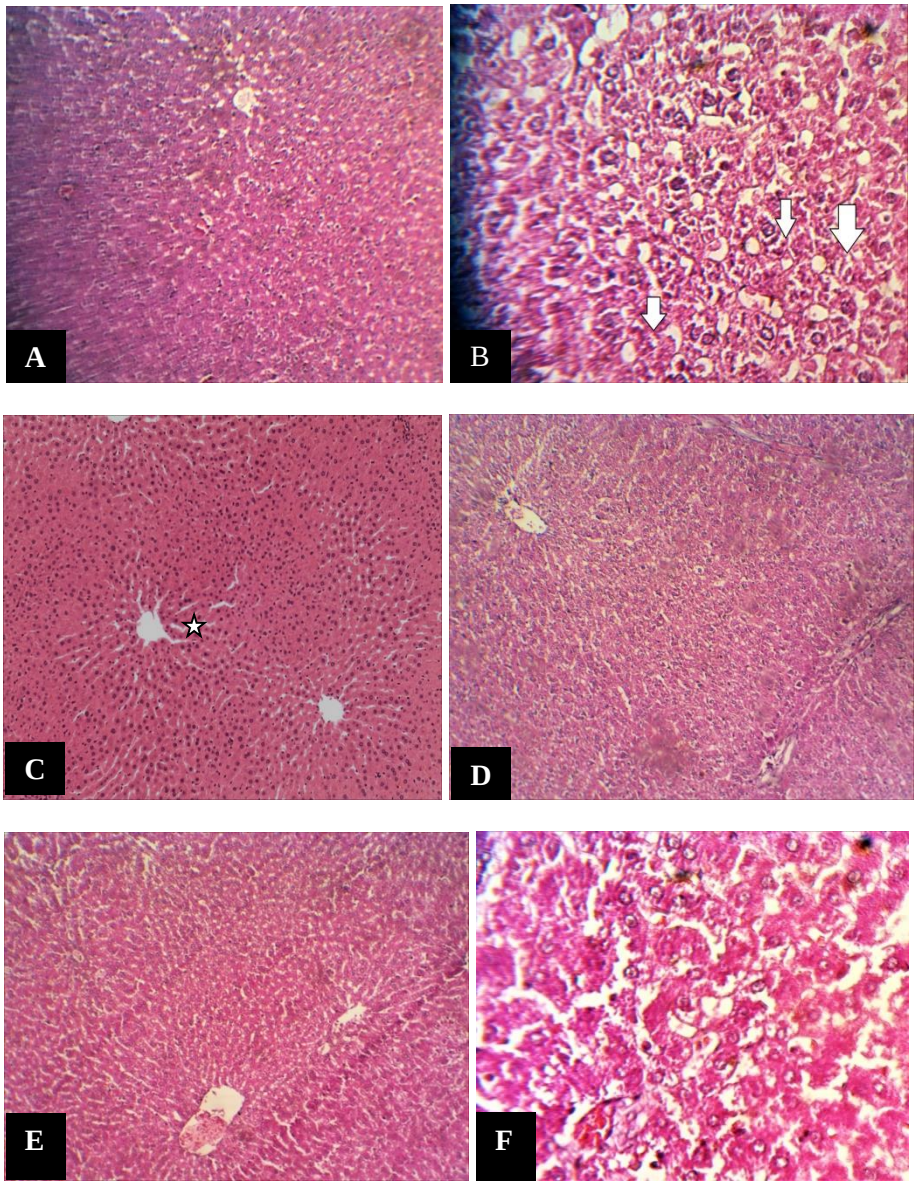
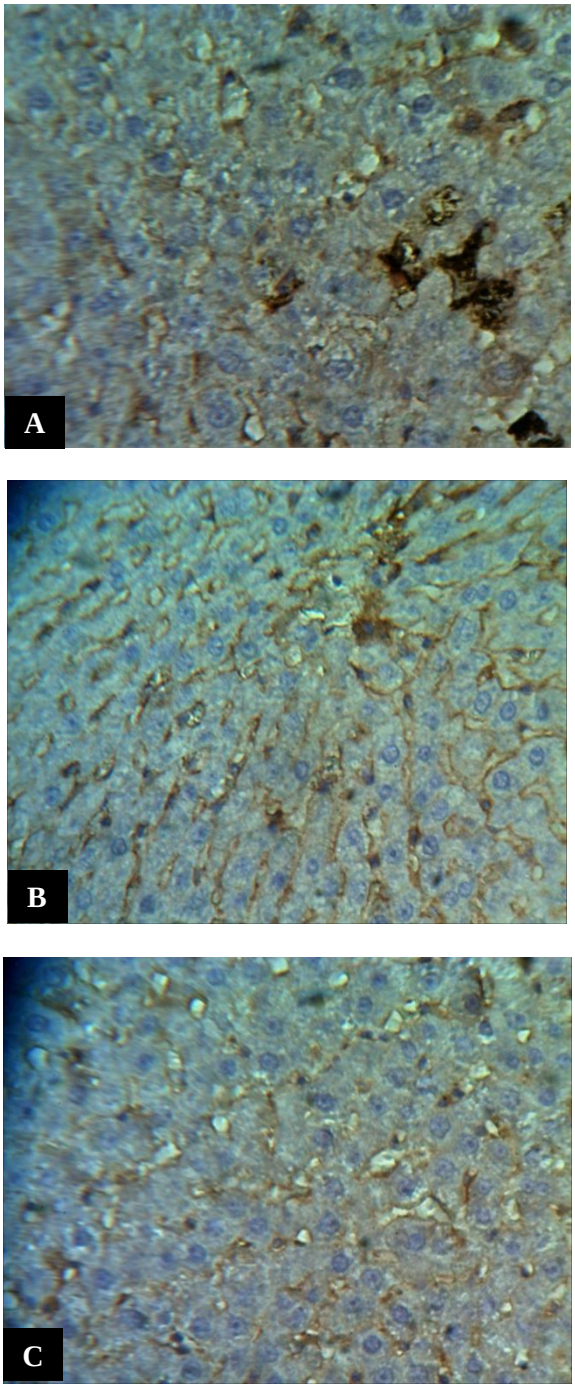


Figure 9B

A- *Liver C200 KI-67 400x.* ***B-*** *Liver Fatty KI-67 400x.* ***C-*** *Liver Normal KI-67 400x*



A- LIVER – NORMAL (H&E 100x)

- NO PATHOLOGICAL CHANGE
- KI-67: 3%

B- FATTY LIVER (H&E 400x)

- STEATOSIS IS PRESENT (++) in which showing hepatocytes with variable cytoplasmic vacuolation (arrow).
- NECROSIS: MILD PRESENT (+)
- KI-67: 8%

C- Ginger-treated Group (C200) (H&E 100x)

- NO PATHOLOGICAL CHANGE
- NECROSIS: MILD PRESENT (+)
- KI-67: 10%

D- Fatty Liver with Ginger C200 (H&E 100x)

- STEATOSIS IS PRESENT (++)
- NECROSIS: NEGATIVE (-)
- KI-67: 14%

E- FATTY C200 - 8 WEEK (H&E 100x)

- STEATOSIS : MILD PRESENT (+)
- NECROSIS: NEGATIVE (-)
- KI-67: 6%

Figure 10A shows the histopathological analysis of testicles after animals have been treated with the extracts. In normal control, complete differentiation of seminiferous tubules with regular cell arrangement is observed (group I), it was evident in the tissue of the other treated groups without changes in morphological.

Figure 10A

The Photomicrographs of Testes Section of all Groups Showing: Normal Architecture (H&E 100x)

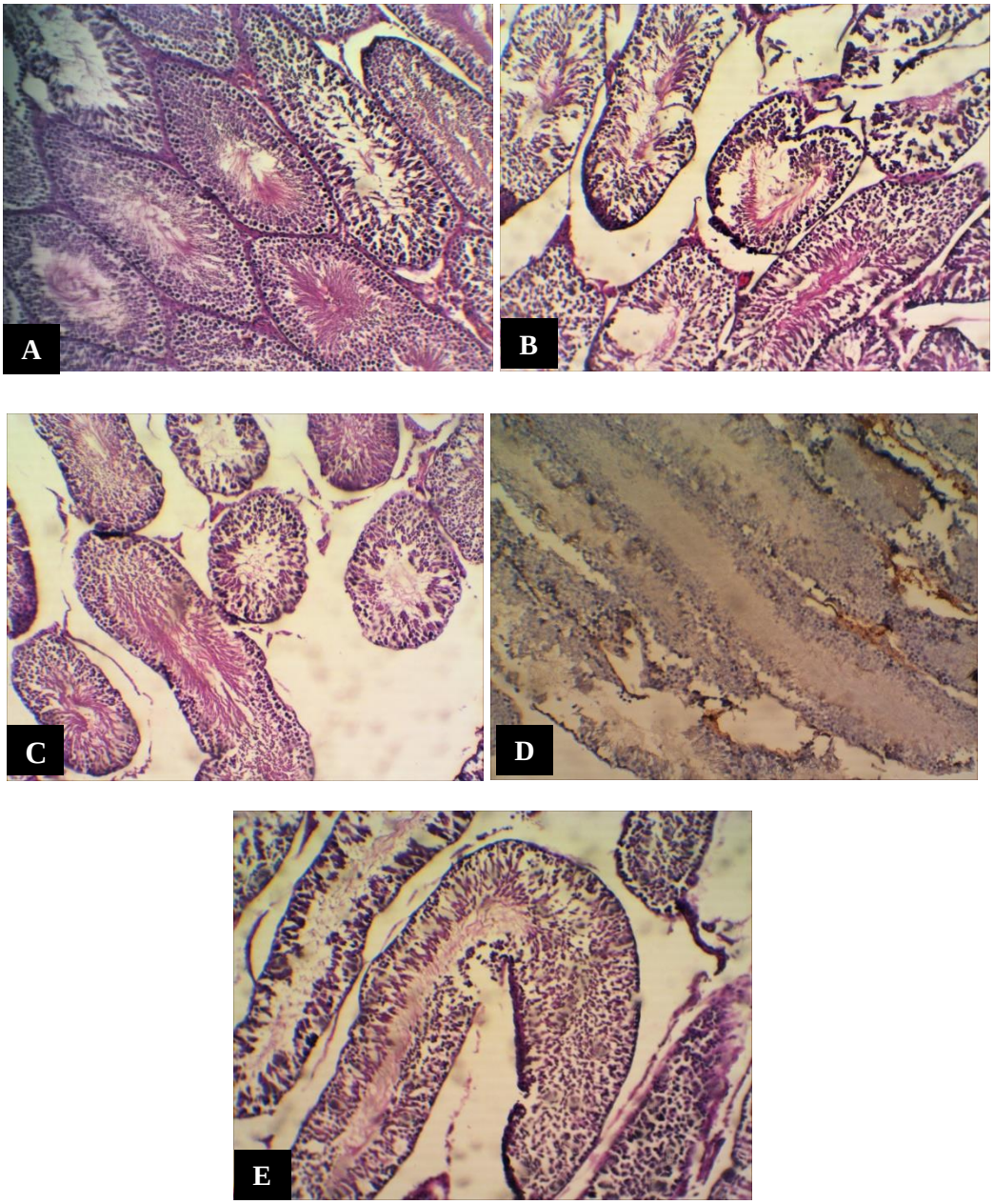
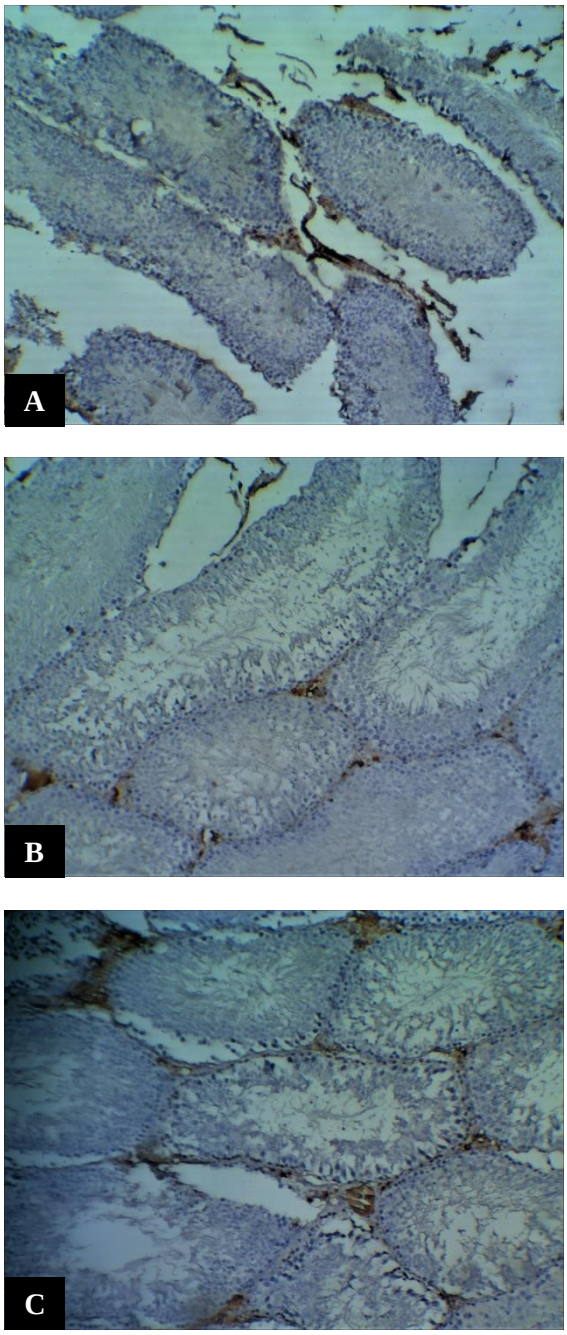


Figure 10B

A- *Testis Fatty C200 8 week KI-67 100x.* ***B-*** *Testis C200 KI-67 100x.* ***C-*** *Testis Normal KI-67 100x*



A- TESTICLES - NORMAL
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

B- TESTICLES – FATTY LIVER
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

C- TESTICLES – C200
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

D- TESTICLES – FATTY C200
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

E- TESTICLES – FATTY C200 - 8 WEEK
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

Figure 11A shows the histology results of heart sections with a normal presentation for the control and ginger-treated groups (groups I&III), but with a small accumulation of lipid droplets infiltrating the elastic walls in high-fat diet group (group II) and elastic connective tissue thickening. Cardiac muscle tissues revealed normal histological appearance in (group I&V).

Figure 11A

The photomicrographs of Cardiac Muscle Section of All Groups Showing Normal Tissues with Long, Wavy Smooth Muscle in Uniforms Arrangement (H&E 100x)

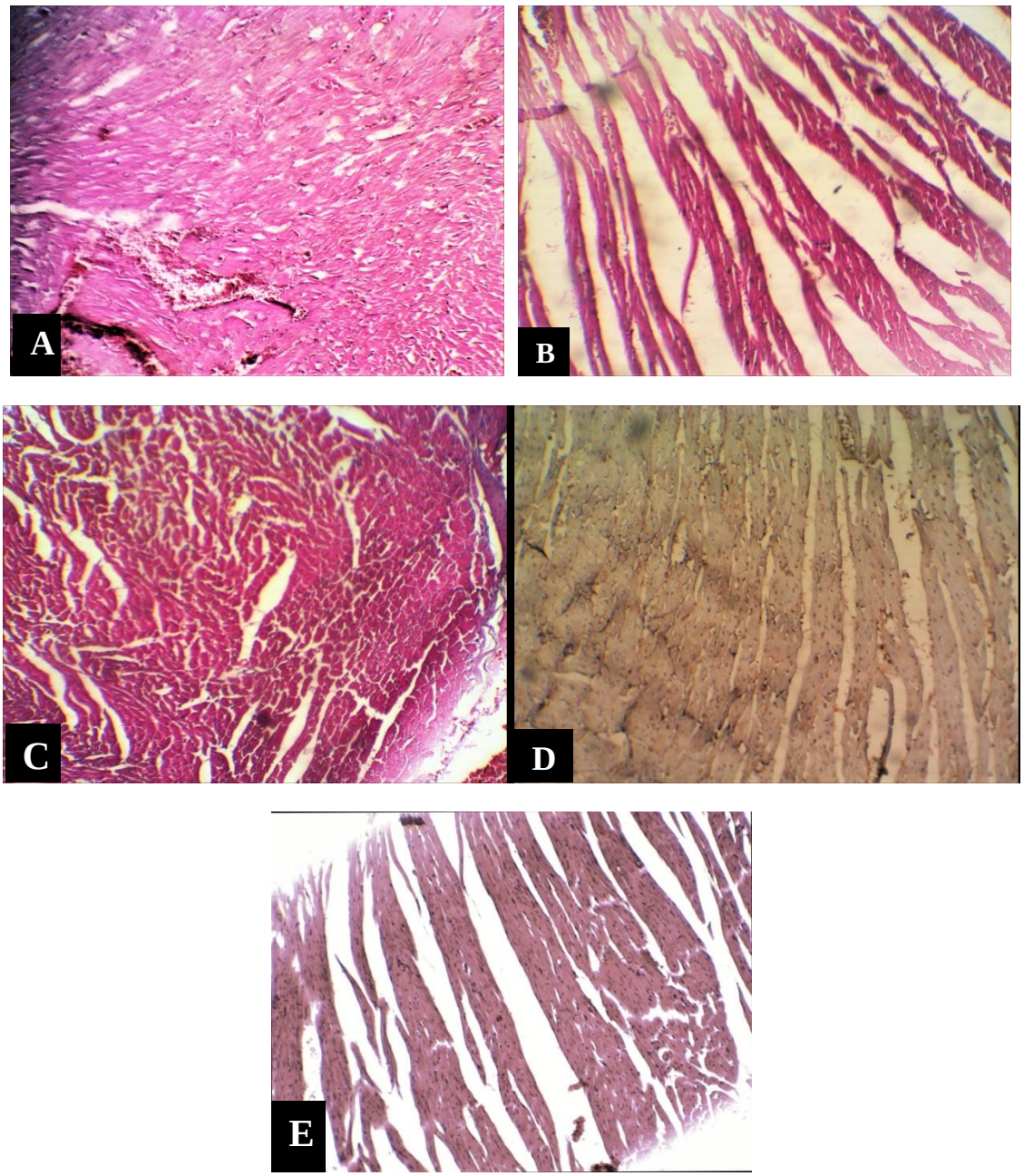
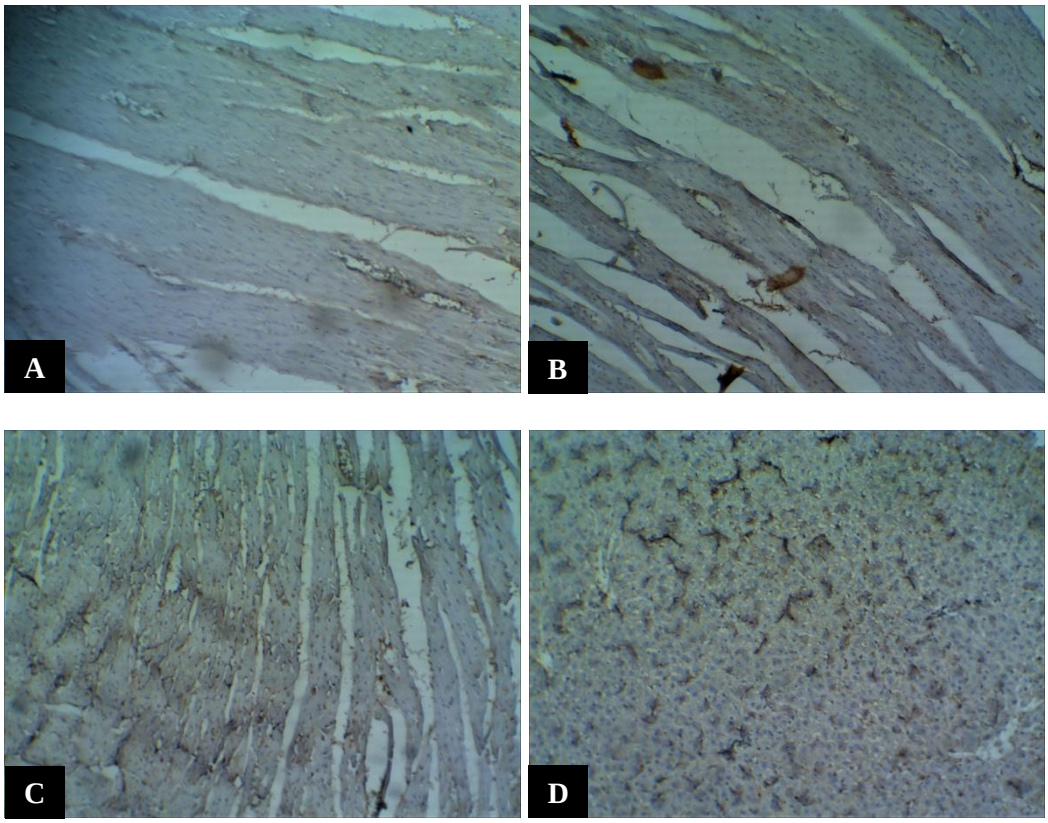


Figure 11B

A- *Heart Normal KI-67 100x.* ***B-*** *Heart Fatty KI-67.* ***C-*** *Heart C200 KI-67.*

D- *Heart Fatty 8 week C200 KI-67 100x*



A- HEART - NORMAL
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

B- HEART – FATTY LIVER
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

C- HEART – C200
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

D- HEART – FATTY C200
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

E- HEART – FATTY C200 – 8 WEEK
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

Light microscopic examination revealed normal renal corpuscles of renal cortex sections of the control and other groups. The renal corpuscle consisting of a glomerulus surrounded by visceral and partial layers of Bowman's capsules which were separated by Bowman's spaces (Figure 12A).

Figure 12A

Photomicrographs of Kidney Sections of All Groups Showing a Glomerulus Surrounded by Visceral and Partial Layers of Bowman's Capsules (H&E 100x)

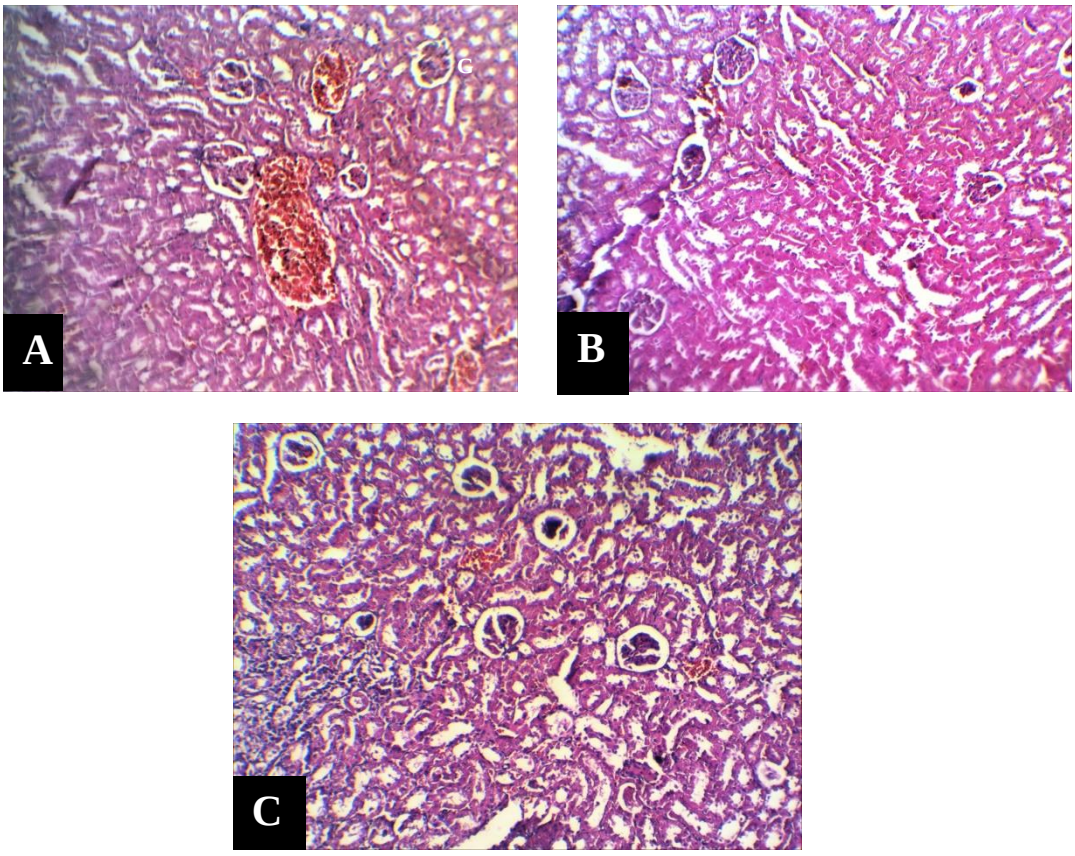
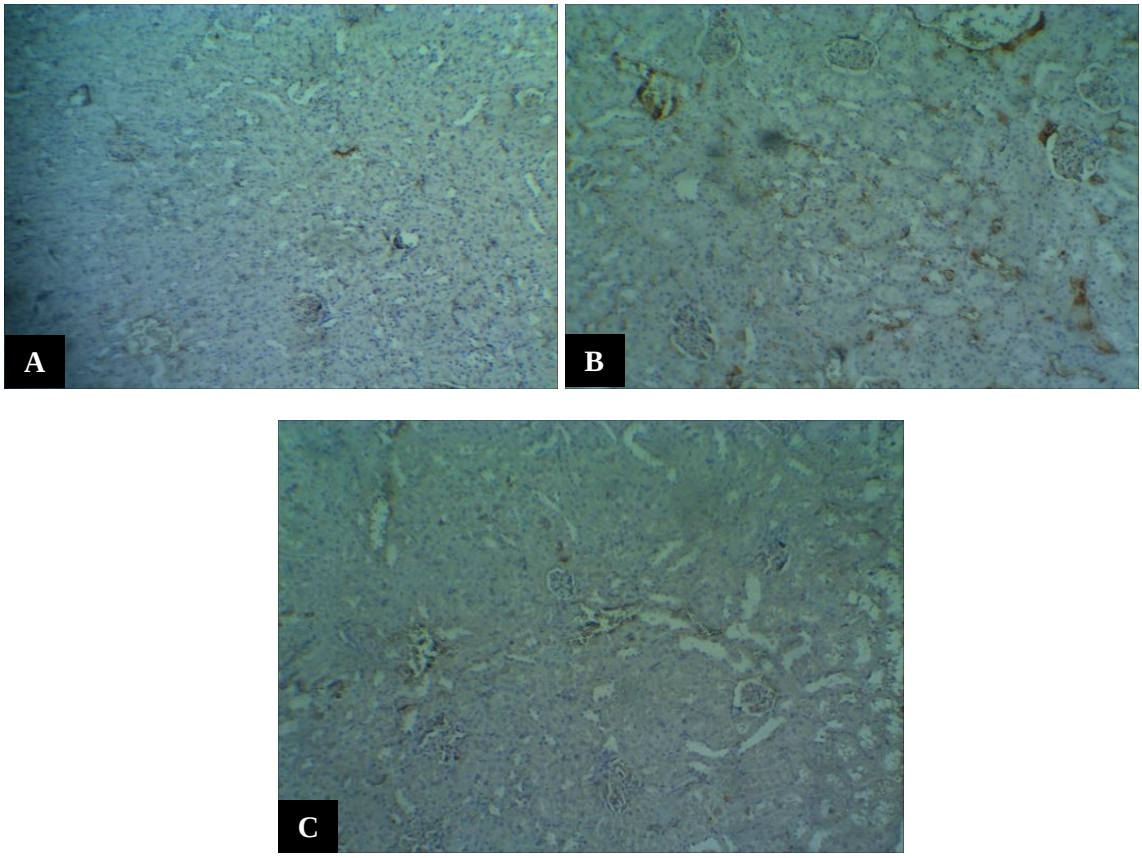


Figure 12B

A- *Kidney Normal KI-67 100x.* ***B-*** *Kidney C200 KI-67 100x.* ***C-*** *Fatty Liver Kidney KI-67 100x*



A- KIDNEY - NORMAL
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

B- KIDNEY – FATTY LIVER
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

C- KIDNEY – C200
- NO PATHOLOGICAL CHANGE
- KI-67: 2%

D- KIDNEY – FATTY C200
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

E- KIDNEY – FATTY C200 – 8 WEEK
- NO PATHOLOGICAL CHANGE
- KI-67: 0%

CHAPTER IV

Results and Discussion

Ginger commonly known as ‘ginger’ has the origin which goes back to Asia. It has many medicinal uses when it comes to herbal medicine. Furthermore, consumption of ginger rhizome is a typical traditional remedy for relieving common health problems such as pain, vomiting, and nausea (Li, et al., 2019). In particular, a significant number of Randomized Clinical Trial (RCT) created to examine antiemetic effect of the ginger in many conditions such as motion sickness, pregnancy, and post-anesthesia (Weimer, et al., 2012; Ensiyeh & Sakineh, 2009; Sharifzadeh, et al., 2018). It is proven that the ginger has anticonvulsant, anti-inflammatory, antidiuretic, antihypertensive, antidiuretic, antispasmodic, antifungal, antitumor and anticancer properties. Ginger contains essential oils (~1% to 3%), pungent non-volatile components and oleoresins (Zick, et al., 2008). Various active components have been identified in the resin oil of ginger, including gingerols and shogaols (Ali, et al., 2008; Mahomoodally, et al., 2019). Since ginger extracts contain various components, it will be important to determine which compounds are responsible for their pharmacological effects. It has been shown that 6-, 8- and 10-gingerols and 6-shogaol show activity in anti-inflammatory, antibacterial, antilipidemic, antipyretic, antitumorigenic and antiangiogenic effects (Park, et al., 2008).

The liver and kidney are important vital organs in the animal body as they are places where toxic substances are detoxified and eliminated. A foreign body in the form of chemical stress is sufficient to cause severe liver and kidney dysfunction (Waggas, 2013). The liver has been identified as the main site of pyrethroid metabolism (Rickard *et al.,* 1985). The accumulation of triglycerides in hepatocytes is defined as fatty liver disease (Kleiner, et al., 2005). Fatty liver has become an important and common liver disease worldwide (Targher, et al., 2010) and increases the risk of death. The pathological picture of fatty liver differs from the presence of small droplets of cytoplasmic fat in hepatocytes (Szczepaniak, et al., 2005). Hepatocyte ballooning, inflammatory infiltrates, apoptosis, collagen deposition and finally liver cirrhosis (Targher & Arcaro, 2007). The antioxidant properties and cholesterol and triglyceride lowering effects of ginger were reported by Helal and his colleagues (Helal, et al., 2012). Previous studies have demonstrated that giving rats a diet containing

ginger (1%) for four consecutive weeks was sufficiently effective to ameliorate hepatotoxic effects (Ajith, et al., 2007; Mallikarjuna, et al., 2008).

One of the distinctive features that separate NAFLD from NASH is the existence of hepatocyte damage. In the NAFLD setting the hepatocytes can be damaged by many different mechanisms. Hepatic fibrosis development in NASH indicates a poor outcome. Histological analysis typically demonstrates steatosis, lobular inflammation, hepatocyte ballooning in acinar region 3, and the microcirculatory unit where blood exits the liver (Kleiner & Brunt, 2012). Fibrosis results and predicts mortality better (Angulo, et al., 2015). Currently, no pharmaceutical treatment has been confirmed for the NASH. For obese and selected NASH patients, bariatric surgery is a good option because it often improves NASH (Lassailly, et al., 2015).

In the present study, the rats in high-fat diet group has significantly ($p<0.05$) increased level of the total triglyceride, cholesterol, SGOT, SGPT and alkaline phosphatase were observed, with a non-significant ($p<0.05$) increase in LDL and HDL among fatty liver induced groups as compared with the control groups (Histogram 1.4 A&B). The results are in line with Altunkaynak's observations with his colleagues (Altunkaynak, et al., 2008).

In this study, the group that received 200 mg of ginger extract, revealed a statistically significant ($p<0.05$) increase of alkaline phosphatase, SGOT and SGPT level (Histogram 1.3 A&B), with non-significant ($p<0.05$) small effect on cholesterol, triglyceride, HDL and LDL levels shown in this group regarding the control group (Histogram 1.1, 1.2 and 1.4).

The decrease in LDL levels of rats treated with the extract compared to the high-fat diet group is an indication that ginger extract may have protective effects on biochemical markers of hyperlipidemia. The protective effects of the ginger in improving markers of hyperlipidemia may be attributed to the radical scavenging antioxidant polyphenols that present in ginger (Kim, et al., 2018). The diet-induced obesity model in the rat is well controlled and shares many features with human obesity. High cholesterol diet and saturated fat has been shown to promote atherosclerosis (Hsu, et al., 2007). However, ginger consumption also stimulates the heart muscles, resulting in better blood circulation in the body and increased cellular metabolic activity. It also helps reduce blood pressure and cardiac workload (Shoji, et al., 1982).

The results of the group IV and V rats that induced fatty liver plus ginger, revealed a statistically significant ($p<0.05$) decrease of alkaline phosphatase, with significant ($p<0.05$) decrease of the cholesterol, triglyceride, SGOT and SGPT levels (Histogram 1.1, 1.2 and 1.3), whereas, non-significant ($p<0.05$) decrease of the HDL and LDL levels revealed among the group that induced fatty liver plus ginger extract compared with the fatty liver group (Histogram 1.4 A&B). In the ginger treatment group the results of the serum lipid profile seem to support the protective role of this extract.

Similar results were noted by Kassaee and colleagues, who suggested that the plant extract may plays an important role in improving liver functions and may also have a protective effect against hepatocellular damage (Kassaee, et al., 2017).

Results of this study shows that feeding rats a high-fat diet resulted in significant increase in serum Triglyceride (TG) and Total Cholesterol (TC) levels and a significant decrease in the HDL-C when compared to the control group. These results can be explained on the basis that rats fed a high-fat diet had an increase in cholesterol absorption and thus an increase in serum cholesterol and triglycerides (Lim, et al., 2013). Hyperlipidemia may also be due to a reduction in catecholamines leading to reduced ß2-adrenergic receptor function and decreased lipolysis, which helps to reduce fat catabolism and increase circulating lipid levels (Al-Awadi, et al., 2013). In this study, the reduction in HDL cholesterol in animals fed a high-fat diet can be due to a decrease in the enzyme involved in cholesterol transesterification, maturation of HDL, and efflux of cholesterol from cell membranes to HDL (Shepherd, 1994).

In our study, there were no significant differences in the kidney, testes and heart tissues when compared with control group. The reason for extension from week 5 to week 8 was to see the effect and change between weeks, however, no effect or change were seen.

4.1 HISTOLOGICAL STUDY

Depending on the purpose of the biopsy, the method of preparing the tissue may vary (Van, et al., 2018). Histological examination of liver sections obtained from fatty liver induced group (Group II) revealed several histological changes in the form of disturbed hepatic architecture (Figure 9A: B). Most of the hepatocytes showed variable degrees of cytoplasmic vacuolations, some contained multiple small vacuoles the others appeared ballooned with peripheral nuclei.

This study noted a significant increase in weight of high-fat fed animals comparing with the control group that may be due to the deposition of fats in various body fat pads. Fidèle and his colleague (Fidèle, et al., 2017) found that cholesterol deposition caused an increase in the rate of production of steroid hormones such as cortisol, estrogens and testosterone, which led to an increase in body weight in HFD-fed rats. In addition, the overgrowth of adipose tissue involves two growth mechanisms that cause obesity first, hyperplastic and second is hypertrophic (Jo, et al., 2009).

The liver is an important vital organ in the animal body as it is the place where toxic substances are detoxified and excreted. A foreign body in the form of chemical stress is sufficient to cause severe liver and kidney dysfunction (Waggas, 2013). The liver has been identified as the main site of pyrethroid metabolism (Rickard, et al., 1985). The accumulation of triglycerides in hepatocytes is defined as fatty liver disease (Kleiner, et al., 2005). Fatty liver has become an important and common liver disease worldwide (Targher, et al., 2010) and increases the risk of death. The pathological picture of fatty liver differs from the presence of small droplets of cytoplasmic fat in hepatocytes (Szczepaniak, et al., 2005). Hepatocyte ballooning, inflammatory infiltrates, apoptosis, collagen deposition and finally liver cirrhosis (Targher & Arcaro, 2007). The antioxidant properties and cholesterol and triglyceride lowering effects of ginger were reported by Helal and his colleagues (Helal, et al., 2012).

In this study, several histopathological changes were seen in the liver of HFD-fed animals, the hepatocytes undergo hydropic degeneration and become swollen and vacuolated, described as ballooning degeneration. The previous studies reported that the consumption of HFD may play an important role in fatty liver pathogenesis resulting in hepatocellular damage and exaggerated hepatic steatosis that is associated with the other factors such as

oxidant stress, mitochondrial injury, fatty acids lipotoxicity and inflammatory cytokines (Hassan, et al., 2018; Aborhyem, et al., 2016).

Hasan and coworkers also found that fatty liver were induced in a group of rats that for 6 weeks fed a high-fat diet, resulting in histological results such as impaired hepatic structure, occlusion and dilation of central vessels, blood sinusoids, and portal vessels (Hassan, et al., 2018).

The histologic spectrum of NAFLD contains a large and small droplet macrovesicular steatosis together or apart portal and lobular inflammation, and steatohepatitis characterized with inflammation, cell damage and steatosis namely Nonalcoholic Steatohepatitis (NASH). It is thought that 10-15% of NASH patients develop fibrosis progression and architectural remodeling and 15-25% develop cirrhosis. Therefore, 3-5% of all individuals with fatty liver may develop cirrhosis (Farrell, et al., 2006).

Hepatocellular damage has been explained by two main mechanisms (Wierzbicki, et al., 2012). The direct mechanism involves the direct cytotoxicity of fatty acids on hepatocytes as a result of extreme intracellular fatty acid accumulations, and the indirect mechanism involves the cytotoxic effects of lipid peroxidation of fatty acids (Ma & Li, 2006).

Second, we examined the effect of ginger extract on structural change in the heart and rescued inflammatory responses in cardiac tissue. Previous studies by us and others have shown that the application of ginger protects against abnormalities caused by diabetes and has many beneficial and useful properties (Taghizadeh, et al., 2007; Shanmugam, et al., 2011). The protective effect of ginger is due to its anti-inflammatory and antioxidant properties. Due to the antioxidants that ginger contains, the supplementations of ginger will increase the total antioxidant capacity and reduces the lipid oxidation in the diabetes and other oxidative stress circumstances (Taghizadeh, et al., 2007, Ramudu, et al., 2011). Accordingly, if diabetes causes structural and functional abnormalities through oxidative stress, as previous studies have approved, the effects of the ginger supplementations in correcting these abnormalities will be due to antioxidant properties. It has also been demonstrated that ginger has the anti-inflammatory effects and also suppresses the expression of pro-inflammatory cytokines such as Tumor Necrosis Factor α (TNF-α) (Isa, et al., 2008; Srivastava, et al., 1992). Ginger compounds shogaol and gingerol inhibits the

biosynthesis of leukotriene and prostaglandin through the suppression of 5-lipooxygenase synthetase (Srivastava, et al., 1992).

In a similar study, Uz and colleagues analyzed the possible protective effects of the dietary ginger against damages that caused by Reactive Oxygen Species (ROS) during renal ischemia on thirty rats using histopathological and the biochemical parameters (Hamed, et al., 2012). Adding ginger to the diet prior to ischemic damage resulted in higher total antioxidant capacity and lower levels of total oxidant status compared to the ischemic group. The ginger-supplemented diet before the ischemic process showed a reduction in the histological features of kidney damage. Their results show that ginger exerts renoprotective effects, possibly through antioxidant activities and radical scavenging (Hamed, et al., 2012). Therefore, it is believed that oxidative stress due to abnormal ROS production plays a significant role in the etiology of kidney toxicities (Mehrdad, et al., 2007; Ramudu, et al., 2011).

CHAPTER V

Conclusion

Ginger has been used widely throughout history for its many natural medicinal properties and especially as a hepatoprotective agent. Present study shows that ginger has a protective role against developing nonalcoholic fatty liver and improving the lipid profile. Ginger extract administration caused a significant reduction in lipid accumulation in hepatocytes compared to positive control rats, ameliorating fat changes in the livers of rats. Therefore, according to the data we found ginger ingestion is safe in humans and might be a promising hepatoprotective agent. The reason for the extension from week 5 to week 8 was to see the effect and change between weeks, however, no effect or change was obtained. In addition, both *in vitro* studies and related animal studies are recommended and rationally designed clinical trials are required. Due to the free radical scavenging property of ginger, anti-inflammatory and antioxidant enhancer, it is therefore necessary to suggest that ginger deserves many clinical trials, especially in the high risk patients, for example, chronic alcoholics and the peoples with the liver dysfunction. As a result of this study, ginger might be useful for the further clinical applications in the humans.

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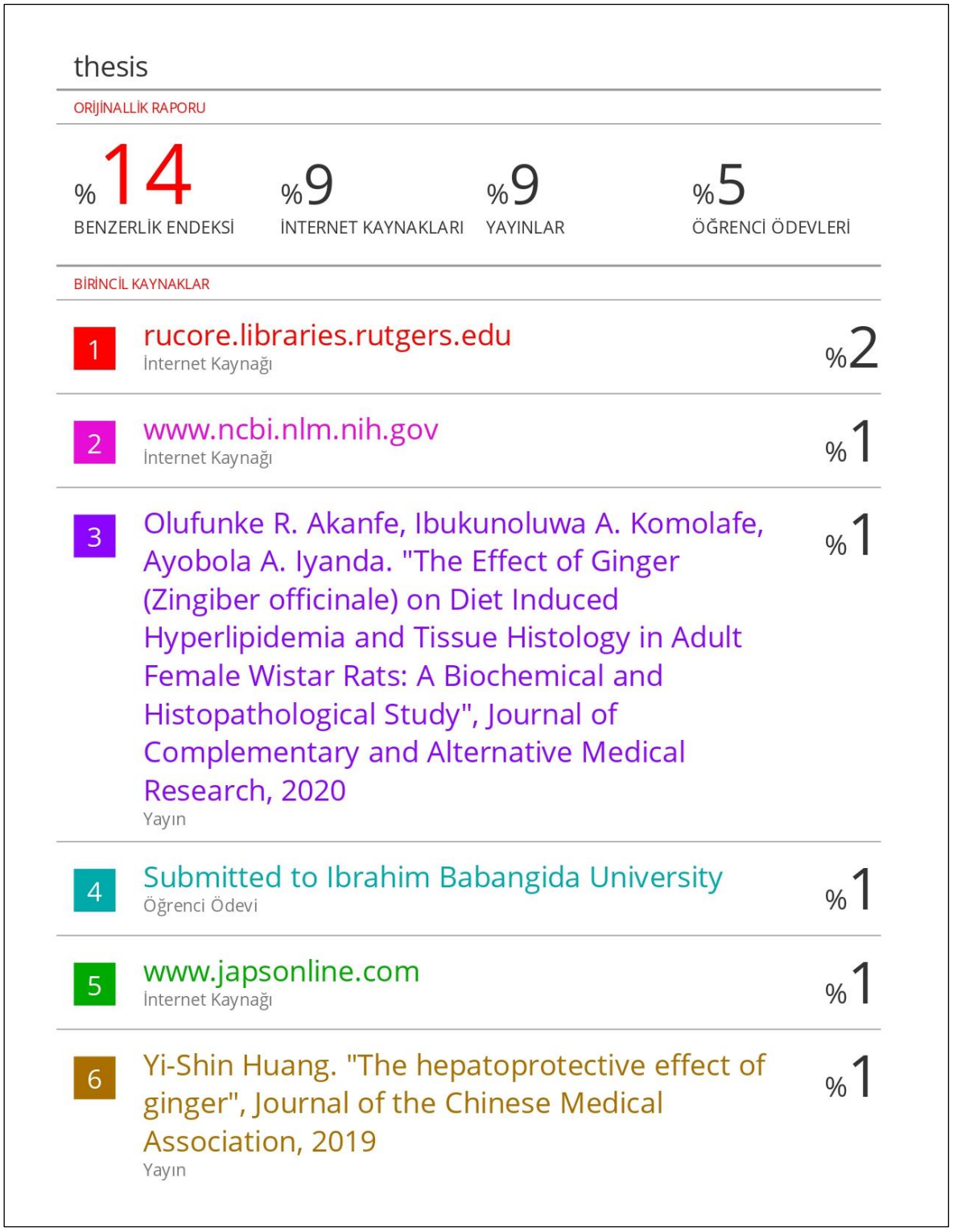
APPENDIX A



thesis

ORİJİNALLİK RAPORU

%14	%9	%9	%5
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	rucore.libraries.rutgers.edu İnternet Kaynağı	%2
2	www.ncbi.nlm.nih.gov İnternet Kaynağı	%1
3	Olufunke R. Akanfe, Ibukunoluwa A. Komolafe, Ayobola A. Iyanda. "The Effect of Ginger (Zingiber officinale) on Diet Induced Hyperlipidemia and Tissue Histology in Adult Female Wistar Rats: A Biochemical and Histopathological Study", Journal of Complementary and Alternative Medical Research, 2020 Yayın	%1
4	Submitted to Ibrahim Babangida University Öğrenci Ödevi	%1
5	www.japsonline.com İnternet Kaynağı	%1
6	Yi-Shin Huang. "The hepatoprotective effect of ginger", Journal of the Chinese Medical Association, 2019 Yayın	%1

APPENDIX B

YAKIN DOĞU ÜNİVERSİTESİ
Sağlık Bilimleri Enstitüsü
YÖNETİM KURULU KARARLARI

Toplantı Tarihi	Toplantı Sayısı	Karar Sayısı
04.02.2020	**153**	**SBE /2020-153-29 a,b**

Karar: 29

"Histoloji ve Embriyoloji" Yüksek Lisans öğrencileri:

a) **Ibtehal Abulgassem ALBADRI (20185196)**'nin ve
b) **Harez Jawdat Jaafar KABANCHI (2085638)**'nin tez önerileri görüşüldü.

a) **Ibtehal Abulgassem ALBADRI (20185196)**'nin **"The effect of Chenopodium quinoa saponins on the proliferation of MCF-7 and MDA-MB-231 breast cancer cells"** konulu tezini Prof.Dr. Aysel KÜKNER danışmanlığında ve Doç.Dr. Pınar TULAY eş danışmanlığında yürütmesine oybirliği ile karar verildi.

b) **Harez Jawdat Jaafar KABANCHI (2085638)**'nin **"Histological, Biochemical and Immunohistochemical Study of Zingiber officinale on experimentally Induced Fatty Liver in Rat"** konulu tezini Prof.Dr. Aysel KÜKNER danışmanlığında ve Doç.Dr. Twana A MUSTAFA eş danışmanlığında yürütmesine oybirliği ile karar verildi.

(İlgi: Histoloji ve Embriyoloji Anabilim Dalı Başkanı Prof.Dr. Aysel KÜKNER'in 2020/1 sayılı ve 22.01.2020 tarihli yazısı.)

ASLI GİBİDİR

Prof. Dr. K. Hüsnü C. BAŞER (Md.)		Prof. Dr. Nazmi ÖZER (Üye)	
Doç.Dr. Kerem TERALI (Md.Yard.)		Prof. Dr. İhsan ÇALIŞ (Üye)	
Doç.Dr. Umut AKSOY (Md.Yard.)		Prof. Dr. Ümran DAL YILMAZ (Üye)	
Prof.Dr. Mehtap TİRYAKİOĞLU (Üye)		Prof.Dr. Adile ÖNİZ ÖZGÖREN (Üye)	
Doç. Dr. Beyza H. ULUSOY (Üye)			

Prof. Dr. K. Hüsnü Can BAŞER
Müdür
Sağlık Bilimleri Enstitüsü

APPENDIX C

Republic Of Iraqi Federal/ Kurdistan Regional Government
Ministry Of Higher Education & Scientific Research
Erbil Polytechnic University
Vice President Office for Scientific affairs

زانکۆی پۆلیتەکنیکی هەولێر
ERBIL POLYTECHNIC UNIVERSITY

Support Letter

Decision Concerning the Scholar´s Research

We support (Harez Jawdat Jaafar Kababchi) for her scientific research entitled (Histological,Biochemical and Immunohistochemical study of zingiber officinale on experimentally induced fatty liver in rat) regarding the necessity of using a sample of (Male Adult Wistar Albino Rat). The researcher needs this sample both alive and dead for her research. The demise of this animal is part of the research requirement and the researcher has legal right to do so according to the Iraqi Animal Protection Law (No. 17), Article 8, on 15/2/2010 which exempts scientific researchers of the Ministry of Scientific Research from the rules of this regulation and permits them to do their researches and studies accordingly.

Dr.Nageb Toma Rassam
Vice. President for Scientific Affairs
Erbil – Iraq

Ministry of Higher Education & Scientific Research
Vice President for Scientific Affairs
Erbil Polytechnic University

E.mail :nageb.rassam@epu.edu.iq Mobil : 0750 4611444 Erbil Governorate, beside MOHE

CURRICULUM VITAE

HAREZ JAWDAT JAAFAR

City: Erbil
Date of Birth: 24.09.1992
Mobile: +964 750 769 0300
E-mail: Harezjawdat@gmail.com

PERSONAL STATEMENT

I am energetic, flexible and ambitious, ready to approach any task I have to undertake or the situation presented to me. I am hardworking and punctual, well organized and can work under pressure.

SKILLS

- Attention to details.
- Able to work on tasks individually or as part of a team.
- Attended health education seminars.
- Patient advocacy.
- Problem-solving skills.
- Good interpersonal skills.
- Communicative.
- Computer literacy (Microsoft Word, Excel, PowerPoint etc.).

LANGUAGES

English: Very Good **Turkish**: Fluent

Kurdish: Fluent **Arabic:** Very Good

EDUCATION & QUALIFICATIONS:

2018 Bachelor degree in Medical Laboratory, Erbil Polytechnic University, Erbil Health Technical Collage.

WORK EXPERIENCE:

- Intern in ERBIL CENTRAL LABORATORY in Bacteriology department, Hematology department, Parasitology department and Biochemistry department 2016-2017.
- Intern in NANAKALI HOSPITAL 2017-2018.
- Intern in BLOOD BANK (Directorate of Blood Transfusion Center/Erbil) 2017-2018.
- Intern in RAPARIN HOSPITAL 2017-2018.
- Intern in ERBIL MATERNITY AND PEDIATRIC HOSPITAL 2017-2018.
- Intern in ERBIL CENTRAL LABORATORY 2017-2018.
- Assistant Lecturer at Shaqlawa Polytechnic University, 2020-2021

CERTIFICATES:

- First aid Course (GLOBAL SURGICAL AND MEDICAL SUPPORT GROUP), 2019 - Erbil.
- English Language Course (LFU), 2020-Erbil.
- Research Excellence Workshop (KScien) organization for scientific research, 2017-Erbil.
- The Web of Science Seminar, 2019, Cyprus International University, Cyprus.