T.C.
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SAĞLIK BİLİMLERİ ENSTİTÜSÜ

EVALUATION OF KALLISTATIN AND SOME BIOCHEMICAL PARAMETERS IN RATS WITH EXPERIMENTAL LIVER INJURY

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İÇ HASTALIKLARI ANABİLİM DALI
(VETERİNER PROGRAMI)
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Tez hakkında alınan jüri kararı, Van Yüzüncü Yıl Üniversitesi Sağlık Bilimleri Enstitüsü Yönetim Kurulu tarafından onaylanmıştır.

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SAĞLIK BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜ'NE

Doktora tezi olarak hazırlayıp sunduğum *"Evaluation of Kallistatin and Some Biochemical Parameters in Rat with Experimental Liver Injury"* başlıklı tezim; bilimsel ahlak ve değerlere uygun olarak tarafımdan yazılmıştır. Tezimin fikir/hipotezi tümüyle tez danışmanım ve bana aittir. Tezde yer alan deneysel çalışma/araştırma tarafımdan yapılmış olup, tüm cümleler, yorumlar bana aittir. Bu tezdeki bütün bilgiler akademik kurallara ve etik ilkelere uygun olarak hazırlanıp, bu kural ve ilkeler gereği, çalışmada bana ait olmayan tüm veri, düşünce ve sonuçlara atıf yapılmış ve kaynak gösterilmiştir.

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TEŞEKKÜR

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SYMBOLS AND ABBREVIATIONS

ALB	: Albumin
ALP	: Alkaline phosphatase
ALT	: Alanine transaminase
ANOVA	: Analysis of variance
APPs	: Plasma proteins called acute phase proteins
AST	: Aspartate aminotransferase
BSEP	: Bile salt export pump
BUN	: Blood urea nitrogen
CBC	: Complete Blood Counts
CCl_3^-	: Trichloromethyl free radical
CCl_4	: Carbon tetrachloride
CK	: Creatine Kinase
CL	: Caudate lobe
COL-I	: Type I collagen
COL-III	: Type III collagen
CT	: Computed tomography
DBIL	: Direct bilirubin
DCL	: Dorsal caudate lobe
DRL	: Dorsal right lobe
ECM	: Extracellular matrix
EDTA	: Ethylenediamine tetraacetic acid
ELISA	: Enzyme-linked immunosorbent assay
FL	: Falciform ligament
GAGs	: Glycosaminoglycan's

GFAB : The glial fibrillary acidic protein

GGT : Gamma-glutamyl transferase

GLB : Globulin

H&H : Hemoglobin and Hematocrit

HA : Hyaluronic acid

Hb : Hemoglobin

HBS : Hepatobiliary scintigraphy

Hct : Hematokrit

HSCs : Hepatic stellate cells

IBIL : Indirect bilirubin

ICV : Inferior cava vein ligated

LDH : Lactate dehydrogenase

LLL : Left lateral lobe

LML : Left middle lobe

LYM : Lymphocyte

LYM : Lymphocyte

MCH : Mean cell hemoglobin

MCHC : Mean corpuscular hemoglobin concentration

MCV : Mean corpuscular volume

MIR : Magnetic resonance imaging

ML : Median lobe

MON : Monocyte

MPV : Mean platelet volume

MRI : Magnetic resonance imaging

N : Number

NEU : Neutrophil

NK	: Natural killer cells
NTCP	: Sodium taurocholate cotransporting protein
OATP	: Organic anion transporting protein
PA	: pre-albumin
PBS	: Phosphate buffer saline
PC	: Paracaval portion
Pct	: Plateletcrit
PIIINP	: Procollagen III amino peptide
PLT	: Platelets
PSS	: Portosystemic shunt
PSSs	: Portosystemic shunts
PT	: Prothrombin time
RBC	: Red cell count
RDW	: Red blood cell distribution
RL	: Right lobe
RML	: Right middle lob
ROS	: Reactive oxygen species
SBA	: Serum bile acid
SGPT	: Serum glutamic-pyruvic transaminase
SIBO	: Small intestinal bacterial overgrowth
SPSS	: Statistical package for the social sciences
STP	: Serum total protein
TBIL	: Total bilirubin
TLR	: Toll-like receptor
UGT	: UDP Glucuronosyltransferase
UGT1A1	: UDP glucuronosyltransferase family 1 member A1

VCL : Ventral caudate lobe

VRL : Ventral right lobe

WBC : White blood cell

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1. INTRODUCTION

The liver, one of the biggest internal organ in the body which performs vital and diverse numerous physiological functions that include metabolism of carbohydrates, proteins, and lipids and takes part in synthesis of immunologic processes, by-products endogenous. Liver also has known as an essential organ which is responsible for of both body and foreign molecules creation, glucose metabolism, protein production and also detoxification of the most exogenous and endogenous toxic chemicals, substance and drugs in the body (Lee et al., 2005; Ničković et al., 2018).

Acute and chronic liver problems occurs frequently with different etiologies which are still not be reducing (Lee et al., 2005; Ničković et al., 2018). Liver hepatocyte injury, presented in acute liver dysfunction of various factors (metabolic malfunctions, viral infections, effectiveness of toxicant agents and etc.), is well-known to be linked with alteration in their enzymatic and non-enzymatic oxidative levels (Ničković et al., 2018).

Chronic liver problems accepted as universal challenge. Liver diseases are considered as a main reason of morbidity and mortality global. Routine medical therapies for these diseases have get limited effectiveness. Whole chronic liver dysfunctions can lead to liver fibrosis and finally hepatic failure as an end stage. So it is worthy to consideration to find new method like new treatment or improve supporting efforts to avoiding of fibrogenesis and hepatic problems (Huang et al., 2014).

Now a days, the exact diagnostic examination of hepatic dysfunctions depends on the histological surveys of liver tissue (liver biopsy) as a process of histopathological examination is considered as a key and proper diagnostic assessment (Lee et al., 2005; Cheng et al., 2015). However, in clinical examination, applying of liver biopsy has numerous risks. For example, sample mistakes, with an estimate mean of more than 20% of false-negative in series of blind liver biopsy processing and other related difficulties (Nord, 1982; Piccinino et al., 1986). Lately, many indirect diagnostic exams for liver dysfunctions have been assessed. Various markers contain clinical symptoms (Piccinino et al., 1986; Tine et al., 1990), endoscopy, blood biochemical parameters (Tine et al., 1990), and ultra-sonographic signs (Giorgio et al., 1986). One of the most

important and useful methods based on liver diseases is laboratory liver tests which are broadly preformed all over the world. As mentioned before, liver performs metabolism of proteins, carbohydrates, and lipids (Gowda et al., 2009). In a case of hepatic related disorders, Compounds derived from the pathway of some metabolic products and some enzymes could be very sensitive and noteworthy in the diagnostic process of liver dysfunction, hence they might be considered as biochemical marker of liver diseases (Gowda et al., 2009).

Accordingly common clinical tests of liver function employs serum analysis of indicators of hepatic disorder, for instance elevates in aspartate aminotransferase (AST) and alanine transaminase (ALT) blood serum levels, midst others (Cheng et al., 2015). Increased serum liver enzyme activities are common in human and veterinary medicine and are, mostly, a diagnostic challenge (Gowda et al., 2009). The complexity of Liver dysfunctions and the influence of numerous environmental factors on the appearance of disease progression and process of responding to therapy, suggest that the necessity of studying on serum based functional to find out markers which cauld be sensitive, precise, diagnostic and prognostic (Cheng et al., 2015). Elevated liver enzymes, a conjugated or separated changing of biochemical markers of liver abnormality in patients are a huge reason of debate among clinicians (Gowda et al., 2009). This serum biochemical enzymes are consider as one of the most common laboratory challenges which can make mistake during the identification of liver diseases (primary belonging to hepatic problems or related to some other parts of the body, secondary due to extra hepatic problems) in this reason it is important to find new diagnostic method to help solving this problem therefore the aim of this study is to explore changing in the level of blood Kallistatin (as a possible specific diagnostic biomarker which is mostly produce in liver) and some biochemical liver indicators like blood serum of ALT, AST, ratio of aminotransferases, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), collagen I (COL-I) and collagen III (COL-III) in rat with experimental liver injury and to determine if serum kallistatin levels could be used as a diagnostic indicator of hepatic healthy Status or not (Gowda et al., 2009; Cheng et al.,2015). In some studies it has been declare that the administration of kallistatin can decrease the progression of liver disease (Jenkins et al., 2010; Huang et al., 2014; Cheng et al., 2015), thus the aim of this study is about changes of the level of the kallistatin and other biochemical parameters in

liver disorders. As a possible result we hypothesized that serum kallistatin levels could be a potential biomarker for liver dysfunctions. In this regards kallistatin serum value can be used to help in other parameters to approach more accurate diagnosis of liver disease and also other investigations based on kallistatin effects. This study can be useful for gathering further information on introduce new biomarker in liver problems in both human and veterinary medicine filed. Besides, it can helpful for studies based on introducing new biomarkers as a clue for diagnosis and treatment of clinical liver diseases (Zhou et al 1992; Wolf et al 1998; Shen et al 2008; Lin et al 2013; Huang et al., 2014; Cheng et al., 2015; li et al., 2015).

2. GENERAL INFORMATION

2.1. Rats Liver

The liver is the largest organ in the body this organ has more biochemical functions amongst other organ of the body which is mainly consisted of hepatocytes, sinusoidal cells, and biliary epithelium (Martins and Neuhaus, 2007; Suckow et al., 2019). The liver has certain functions such as the removal of exogenous and endogenous toxins and synthesis of vital substances like blood clotting agents and albumin. it is also played basic role in metabolism of proteins, fats and carbohydrates, vitamins supply, the proces of conjugation and excretion of bilirubin in bile, activation glycogen and triglyceride and mineral supplies, various hormones, and generating bile salts (Wallace et al., 2008; Jenkins et al., 2010; Huang et al., 2014; Suckow et al., 2019). Hepatocytes make up about 60% of the liver parenchyma with sinusoidal endothelial cells, liver stellate cells (called Ito cells), liver-associated Kupffer cells and lymphocytes (Martins and Neuhaus, 2007; Suckow et al., 2019) (Table 1). The hepatic artery and the portal vein are the two blood supplies of the liver which comprise nearly 20% and 80%, respectively, of the total blood circulation flow, which mixes as it enters the sinusoids. Liver is performs more than 1000 essential biochemical functions. It functions in hundreds of diverse metabolic activities including synthesis of plasma proteins catabolism and storage of carbohydrates synthesis, degradation of lipids detoxification and excretion of many toxic agents the formation and elimination of bile (Martins and Neuhaus, 2007; Higashiyama et al., 2018; Kararli, 1995). Multiple tests have been developed to assess liver function because of this cellular, biliary, and vascular complexity (Martins and Neuhaus, 2007; Suckow et al., 2019). These tests and examinations aim to evaluate hepatocyte function and membrane integrity, the portal circulation, hepatobiliary function, and the enterohepatic circulation. Due to the close anatomic and intertwining functional relationships of hepatic components, there is frequent overlap of findings with varied pathology of the liver and the portal circulation and extrahepatic diseases (Higashiyama et al., 2018; Kararli, 1995).

Table 1. Cells of the liver and their functions (Jungermann and Sasse, 1978; Kozat and Sepehrizadeh, 2017).

Cell Type	Other Name	Function	Cell Markers
Hepatocytes	Liver cells	Intermediary metabolism	Albumin, cytokeratin 8 and 18
Cholangiocytes	Biliary epithelial cells	Line the bile ducts, secretion	Cytokeratin 7 and 19
Kupffer cells	Browicz-Kupffercells, stellate, macrophages	Phagocytosis of pathogens and particles	ED-1 and ED-2
Stellate cells	Ito cells, Vitamin A–storing cells, lipocytes	Storage of vitamin A; production of myofibroblasts in injury	GFAP, desmin; α-smooth muscle actin
Natural killer (NK) cells	Pit cells, large granularlymphocytes, γδ T cel	Immune surveillance infection, cancer	CD3
Vascular endothelial cells	Endothelial cells	Line blood vessels	CD34 and CD31
Lymphatic endothelial cells	Endothelial cells	Line lymphatic vessels	Podoplanin
Smooth muscle cells	Myocytes	Regulation of Microcirculation	Myocardin, α-smooth muscle actin
Stem cells	Progenitor cells, oval cells	Bi-potential progenitor cell for hepatocytes and biliary epithelial cells	α-Fetoprotein
Portal tract fibroblast	Fibroblasts	Integrity of portal triads, supporting function	Vimentin

2.1.1. Anatomy of the rat liver

The rat liver consists of four different lobes of various size: the left lateral lobe (LLL), the median lobe (ML), the right lobe (RL) and the caudate lobe (CL). The largest lobe was the median lobe (Martins and Neuhaus, 2007; Stan, 2018) (Figure 1). Left lateral lobe possess the left section of the epigastric area and the left hypochondriac area and it is uniform. The free part of this lobe is oval shape and surrounding more than half of the stomach and located at ventral of stomach. The left lateral lobe connected to intrahepatic vena cava vein via thin pedicle bound and also

keep in touch with median lobe. The left lateral lobe presented a narrow pedicle bound with the intrahepatic cava vein and a small base attached to the left median lobe (Madrahimov et al., 2006; Martins and Neuhaus, 2007; Stan, 2018).

Median lobe as a biggest lobe of the liver, is found just under the diaphragm, and creates a big section structures of the parietal surface (Madrahimov et al., 2006; Martins and Neuhaus, 2007; Stan, 2018). A profound fissure divides the lobe into two parts, with the large one in the right middle lobe (RML) and the small one in the left middle lobe (LML). This lobe is connected with left lateral lobe (Madrahimov et al., 2006; Stan, 2018).

A large basement of the middle lobe covered approximately whole perimeter of the vena cava. Right lobe is separated by a profound fissure into two overlap sections: the dorsal right lobe (DRL) and the ventral right lobe (VRL). Both of them are found at right hand of vena cava. Entire RL is approximately surrounded by RML and there is a renal sign on VRL (Madrahimov et al., 2006; Stan, 2018). The caudate lobe (CL) is situated close the LLL (almost near the left hand of the vena cava) the whole CL is divided into two sections, one as a dorsal caudate lobe (DCL), and ventral caudate lobe (VCL). The VCL places on the ventral section of the stomach (Madrahimov et al., 2006; Stan, 2018).

The DCL places on the dorsal section of the stomach (beside the pancreas and spleen). Both VCL and DCL are surrounded by lesser omentum. The LLL and the DCL are connected by ligament in to each other. Rats do not have a gallbladder, and each lobe being drained by its own bile duct. The common bile duct is created by primary liver ducts (Madrahimov et al., 2006; Behar, 2013; Stan, 2018; Higashiyama et al., 2018; Kararli, 1995). The falciform ligament is responsible for connecting liver to ventral abdominal wall and diaphragm (Madrahimov et al., 2006; Stan, 2018).

2.1.2. Topography and borders surfaces of rat liver

Rat's liver is located on the cranial abdominal section, and almost semi of liver being found in the intra-thoracic part of abdominal region. The falciform ligament is

responsible for connecting liver to ventral abdominal wall and diaphragm (Madrahimov et al., 2006; Stan, 2018) (Figure 1).

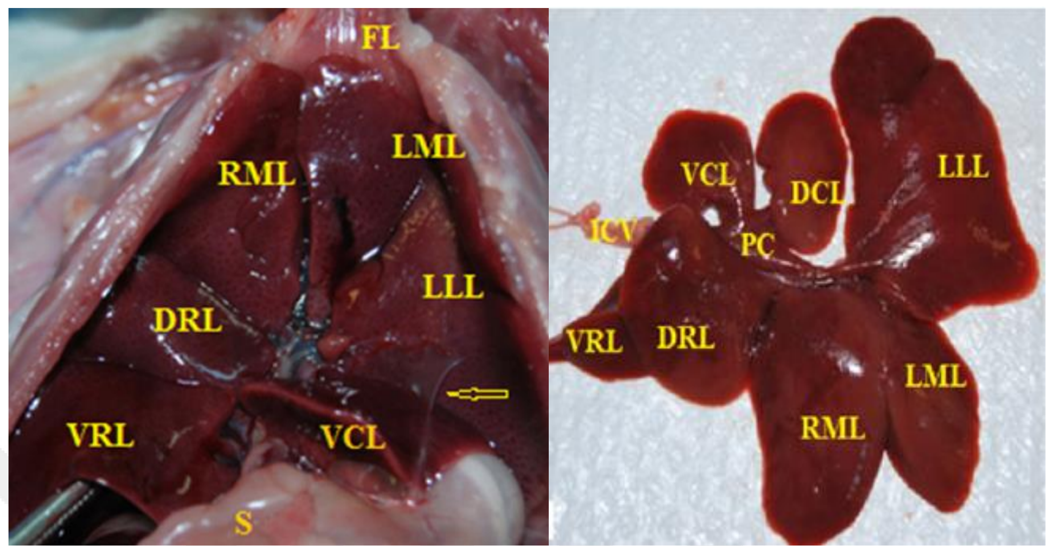

Figure 1. rat liver, LLL-left lateral lobe; LML-left medial lobe; RML-right medial lobe; DRL-dorsal right lobe and VRL-ventral right lobe; FL-falciform ligament; Interlobar ligament-arrow; PC – paracaval portion; VCL – ventral caudate lobe; DCL - dorsal caudate lobe; ICV – inferior cava vein ligated; S-stomach

The caudal borders reach almost to the same surface on both hands of the abdominal region. On the right hand, the caudal border is generated by the RL and on the left hand by the LLL. Additionally, ML, LLL, and RL are placed on the diaphragmatic surface Between the CL, caudal vena cava and the two sheets of coronary ligament, a part of the liver is free and attached to the diaphragm. The visceral surface surrounded entirely by the peritoneum, has contact with stomach and entrails (Madrahimov et al., 2006; Stan, 2018) (Figure 1).

2.1.3. Liver physiology of rat

Hepatic function (Figure 2) declares the circulation of biliary acids, bilirubin and drugs or other substances from the liver cells to the bile and the microanatomic connections of them, the cells place of hepatic enzymes. There is no gallbladder in rats (Madrahimov et al., 2006; Behar, 2013; Stan, 2018). Bile duct is responsible for transport of bile acids into the gut lumen. Most of bile acids are absorbed from distal part of the ileum and again discharged into the bile from the hepatic cells (Uchida et al., 1978; Suckow et al., 2019). At times examining animals with hepatobiliary disease symptoms, clinical

history beside the physical examination can help to formulate a list of reasonable differential diagnosis (Suckow et al., 2019).

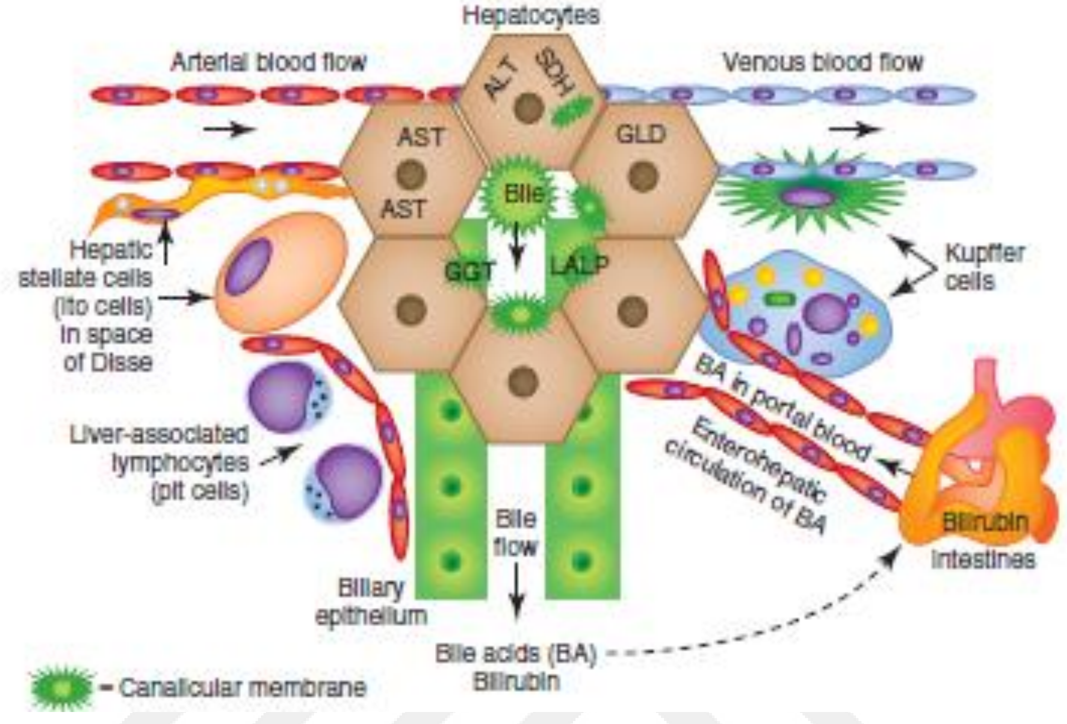

Figure 2. Hepatic functions of rat liver (Kozat and Sepehrizadeh, 2017)

2.2. Diagnosis of Liver Disease

2.2.1. Diagnostic strategy and clinical examination

Diagnostic assessment of the hepatobiliary system has many objectives, for instance to determine if hepatobiliary disease is present, to assess liver function, to definitively diagnose hepatobiliary disease and to monitor response to treatment (Wallace et al., 2008). Obtaining a true history is important to illustrating the clinical related disorders that need to be resolved (Wallace et al., 2008; Suckow et al., 2019) (Table 2.). Fortunately, the knowlede advances in the both medical profession and the veterinary medicine, has increased considerably during the last few years and some basic principles should be memorised to figure out the signs in animal with diseases which affecting the liver parenchyma and portal vasculature (Wallace et al., 2008; Jenkins et al., 2010; Huang et al., 2014). First, the liver has an enormous (almost 85 %) reserve capacity and a

remarkable potential to regenerate. symptom happen just in a case of advanced disease exhausts liver supplies. Problems usually stay subclinical for long periods; symptoms might be mild and nonspecific, because the liver reserves inhibit emergence of abnormalities. Symptoms like vomiting, fatigue, or polyuria and polydipsia may alert the clinician that a liver disorder could be developing (Wallace et al., 2008; Jenkins et al., 2010; Huang et al., 2014; Suckow et al., 2019).

Table 2. Clinical signs associated with liver disease (Kozat and Sepehrizadeh, 2017).

Depression	Weakness	CNS signs, Icterus
Anorexia	Vomiting	Change in spleen size
Diarrhea	Weight loss	Dark brown urine
Fever	Polydipsia	Polyuria
Abdominal pain	Ascites	Coma, Change in liver size
Dark or light color stools	Hemorrhage	Pruritus

2.2.2. Liver dysfunction evaluation biomarkers

a) Direct markers of liver fibrosis

Direct markers are directly played role in removal and deposition of the hepatic extracellular matrix generated by hepatic stellate cell and other hepatic cells. Blood serum levels of these markers are upraised with developing severity of diseases and have an inclination to decline with reaction to therapy (Grigorescu, 2006; Nallagangula et al., 2017). Evaluation of these markers can be beneficial for bringing about efficient therapy, but they are neither organ specific nor easily obtainable. Direct markers of hepatic injury are classified due to their molecular construction (Nallagangula et al., 2017).

Collagens

Liver dysfunction is a wide-spread variation in the liver, which includes initially of elevated deposition of collagen in the tissue (Shiba et al., 1998; Kazeem et al., 2011). Rat liver fresh weight normally includes about 1mg of collagen (Shiba et al., 1998; Kazeem et al., 2011;). Normal livers contains almost equal amounts of type I, type Ill,

and basement membrane collagens. The proportion of type I / type III collagen is less than 1 and it is indicate a slight predominance of type III collagen. In liver diseases apart from etiology, all collagen types are increased (Rojkind et al., 1979). Type I and III collagens in all, making up about 80% of the whole liver collagen, and types IV and V contains about 20% of total liver collagens (Shiba et al., 1998). Definite quantification of collagen types is however hard, and in consequence amounts reported by different labs indicate some contradictions (Rojkind et al., 1979; Shiba et al., 1998). With developing of the severity of hepatic injury the entire liver collagen amount elevates several-fold than normal, and all collagen types are involved (Rojkind et al., 1979; Shiba et al., 1998). Various organs connective tissue consist type I and type III collagens (Rojkind et al., 1979; Shiba et al., 1998). Both types of collagen are also find in scar tissue of skin, tendons, the muscular tissue of the heart, and also in liver (Rojkind et al., 1979; Shiba et al., 1998). There is an obvious connection between type I to III liver collagens which has been declared to stay within normal ranges in the rats with severe liver injury (Ala-Kokko et al., 1987). Whilst type IV and V liver collagens may pro rata elevate even more than type I or III collagens (Ala-Kokko et al., 1987; Shiba et al., 1998). In liver diseases the reason of increasing of liver collagens and tissue accumulations of them are not understood clearly. Several studies have shown that a fibrotic liver generates larger amounts of collagen than a healthy liver (Rojkind et al., 1979; Ala-Kokko et al., 1987; Shiba et al., 1998). But, the content of collagen deposited must rely on the connection between the rates of degradation and synthesis (Rojkind et al., 1979; Ala-Kokko et al., 1987; Shiba et al., 1998). Enzymatic cleavage at carboxy and amino terminal ends on procollagen by procollagen N-peptidase and C-peptidase, is part of synthesis process of collagen (Grigorescu, 2006; Canty-Laird et al., 2011; Nallagangula et al., 2017). Fibril-forming type I collagen is profuse in healthy liver. During fibrogenesis, type I collagen is increased up to eightfold (Gressner et al., 2007). Blood serum evaluations can indicate a sign about severity of liver injury. Fibril-forming type III collagen is a significant part of connective tissue. By progressing of hepatic diseases levels of procollagen III amino peptide (PIIINP) in basement membrane become higher due to chronic hepatic injury. Aminotransferase levels are in relationship with PIIINP in acute hepatic injury which indicates stage of hepatic fibrosis (Gressner et al., 2007; Castera, 2012). Approximately low specificity and sensibility

(81% and 78%) of these markers have restricted their clinical usage. There is no connection amid histological grading of liver fibrosis with PIIINP and procollagen I carboxy peptide blood serum levels. Therefore, these are not trustworthy to determine liver fibrosis grading (Gressner et al., 2007; Canty-Laird et al., 2011; Castera, 2012; Nallagangula et al., 2017).

Hyaluronic acid

Hyaluronic acid (HA) is a glycosaminoglycan's (GAGs) generated by hepatic stellate cells and is the basic part of the hepatic extracellular matrix. In healthy liver, HA absorption and decomposition happen in hepatic sinusoidal endothelial cells. Serum HA quantities are associated to level of fibrosis and level of necrotic inflammation. High levels have been detected in liver fibrosis with varied ethology (Orasan et al., 2016; Nallagangula et al., 2017). By the progressing of liver diseases evaluation of HA as a non-invasive marker of liver fibrosis is worthy to consider and it is one of the golden markers to use as a substitute for liver biopsy in the diagnosis process of liver fibrosis. It has known as diagnostic precision for the noninvasive assessment of liver dysfunctions (Guechot et al., 1996; Parise et al., 2006; Orasan et al., 2016). Hyaluronic acid is a crucial biomarker of the extracellular matrix almost be present in all parts of the body, mainly produced by hepatic stellate cells and decomposed by sinusoidal endothelial cells, which is in relationship with the histological stages of liver fibrosis by progressing of liver diseases (Guechot et al., 1996; Orasan et al., 2016). Elevated concentration in serum are referred to increased construction and reduced hepatic decomposition or both of them (Fouad et al., 2013; Orasan et al., 2016). As the liver injury progressed, the HA specificity and sensitivity reach to 85–100% and 90–95%, respectively (Guechot et al., 1996; Fontana et al., 2009; Orasan et al., 2016).

b) Indirect markers of liver dysfunction

Activation of serum hepatobiliary enzymes needs participation of some important factors, one of them is the normal tissues enzyme concentration. Sufficient amount of Enzyme must be available to handing over into the circulation. The second factor is the half-life of serum enzymes. In order to enzyme serum accumulate, it should have enough half-life duration. The final factor is intracellular localization, Assessment of enzyme serum occurs with the ability of enzymes to access the vascular compartment (Ennulat et al., 2010; Jarsiah et al., 2017; Hasan et al., 2018). On the whole, cytosolic enzymes easily reach to the serum in a compare with enzymes which are membrane-bound or that are presented in organelles. Increasing of Serum hepatobiliary enzyme activity could happen due to elevated synthesis, penetrate from damaged liver cells, elution from injured membrane (Haschek et al., 2013). Evaluation of serum hepatobiliary enzymes activity are helpful screening test for liver diseases. Thanks to their high sensitivity, the false negative result rate is low and not many patients with hepatic disorders are missed. On the contrary, their specificity is low, due to increase false positive results, animals without hepatic disorders could be considered patient. So, once an increasing in serum hepatobiliary enzyme activity is detected, verification of liver disorders needs fulfilling examinations with higher specificity (Ennulat et al., 2010; Jarsiah et al., 2017; Hasan et al., 2018). Alanine aminotransferase (ALT) serum rising are specific remarked in rats. ALT activity can raise with muscle injury, but simultaneous evaluation of Creatine Kinase (CK) activity can be considered in relation to the source of the muscle (Ennulat et al., 2010; Haschek et al., 2013; Jarsiah et al., 2017; Hasan et al., 2018). Raising in serum ALT activity have the highest sensitivity (about %85) for hepatic disorders but have less sensitivity (about 65%) in cases of hepatic congestion, neoplasia, and portosystemic vascular anomalies (Ennulat et al., 2010; Jarsiah et al., 2017; Hasan et al., 2018). Alkaline phosphatase (ALP) serum raising activity is one of the most common problems detected in rats with liver problems. ALP activity measuration has a high sensitivity (85%) for hepatic disorders, but its specificity is low (50%). If elevated ALP activity is noted with a concurrent increase in serum GGT activity, specificity for liver disease increases up to 90 % (Ennulat et al., 2010; Jarsiah et al., 2017; Hasan et al., 2018). In liver disorders, biochemical identification should provide precise diagnostic capabilities that can provide the appropriate protocol to aid further examination (Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al.,

2017; Hasan et al., 2018). Hepatobiochemical abnormalities are usual nonspecific, that is to say the evaluated enzymes could be the same enzyme with another organ origin or isoenzymes from different body places. Biochemical test due to liver abnormalities are divided into three branches (surveys of disruption in metabolic function or liver Synthetic function, hepatocytes injury and cholestasis) (Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). Hepatic enzymes can be divided into markers of hepatocellular damage and markers of cholestasis in animal with liver disease an elevation can happen in both ALT, AST and their activity demonstrate leakage of the enzymes and hepatocellular membrane damage. Liver tissue and skeletal muscles are holding large amount of AST and approximately more than 70 % of AST is found in hepatocyte mitochondria (Kasarala and Tillmann, 2016; Hasan et al., 2018). AST serum rising in the absence of increased ALT activity, show an extrahepatic problems, like muscle injury (Kasarala and Tillmann, 2016; Hasan et al., 2018). Measuring AST activity is sensitive but less specific for detecting hepatic disease than is measuring ALT serum activity (Haschek et al., 2013). As a result of enzyme discharging, permeability of the hepatocytes membrane is changed due to injury or a metabolic abnormality. Measurement serum levels of AST and ALT in a case of heart and the liver problems are important, especially in a way of finding if there is a problem or not (Kasarala and Tillmann, 2016; Hasan et al., 2018). Studies show that, when body tissues are injured, extra AST and ALT are entered into the blood and increase the serum enzymes amount. Consequently, the level of AST and ALT in the bloodstream is in a relation with the level of tissue injury. Increase AST and ALT ratio (more than 1.5) in acute viral liver malfunction could indicate severity of disease (Botros and Sikaris, 2013) (Table 3, 5) Both of ALT and AST levels must be take into account as hepatocytes “leak” enzymes. Following to an acute, diffuse injury, the magnitude of their increasing reflects the number of affected hepatocytes. ALT activity can be further defined as muscular in origin by the measurement of serum CK which is specific muscle enzyme. The serum half-life of ALT is generally believed to be short (less than 8h) (Ennulat et al., 2010) but it might take days to weeks to decrease following an acute injury (Ennulat et al., 2010; Haschek et al., 2013; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). In a case of injury in skeletal muscles, serum AST and ALT will be elevated but its notable that increase amount of ALT is in a much lesser extent. In veterinary medicine clinical experience

indicate that there is value in the explanation of the serum amounts of ALT and AST for liver disease (Ennulat et al., 2010; Haschek et al., 2013; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). Serum ALT and AST levels are the worthy for evaluation of hepatic disorders, while relying on the result of only one enzyme alone cannot be sufficient and both of them must be evaluated (Johansson et al., 2008). As result of acute damaging, there is a moderate marked increase in the serum AST and ALT concentrations, the amount of AST serum will return to normal more rapidly (hours to days) than the ALT serum (days). This happen because of their plasma half-life and cellular location differences (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018).

Serum GGT amount in rats is usually low and may be even hard to detectable in “healthy” animals. Elevated blood plasma GGT in cholestasis could be a result of raised bile acids concentration that stir synthesis and release of GGT like as to ALP (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). The membrane-bound location of these enzymes in the canalicular surface are different, GGT is connected more with epithelial cells of the bile ducts therefore GGT is associated more with the canalicular membrane activities (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). Changes in serum GGT activity usually parallel with serum ALP activity; But, ALP is take into account as less specific but more sensitive indicator of cholestasis when compared with GGT (Haschek et al., 2013). Significant increasing in GGT serum level have importance in problems like cholecystitis and bile duct obstructions (Haschek et al., 2013). Intermediate increases are pointed out inception of liver neoplasia (hepatocyte and biliary carcinoma) (Haschek et al., 2013; Hasan et al., 2018) and mild increases are pointed out liver necrosis (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018).

In the healthy hepatic tissue ALP and GGT show minimum activity, however their serum levels can be raised due to increased enzyme production stimulated by either drug induction or bile flow disorder (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018) (Table 3, 4.). ALT and AST are increasing in a case of hepatocellular leakage whereas ALP and GGT are

increasing in a case of cholestasis. ALP is accepted as a sensitive biomarker for cholestasis with a sensitivity of 85%. Serum elevation of enzymes may sometimes not show liver disorders (Rochling, 2001; Woreta and Alqahtani, 2014) (Table 3, 5.).

Indirect markers as a part of liver biochemistry, beside assessment of liver function are used for recognition of liver diseases as well (Grigorescu, 2006; Nallagangula et al., 2017). Parameters like Gamma-Glutamyl Transferase, Aminotransferases and Alkaline Phosphatase, and assessment of serum total protein (STP), albumin (ALB), serum pre-albumin (PA), albumin/globulin ratio (ALB/GLB), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), globulins (GLB) in blood are considered as indirect biomarkers for liver diseases which are represent variation in liver action and effective in detection, assessing intensity, monitoring treatment as well as evaluating the prognoses of hepatic malfunction (Grigorescu, 2006; Nallagangula et al., 2017). The happening of hepatic disease is the main cause of increased serum transaminase activity due to impaired hepatocytes damage (Kim et al., 2008). Progressing of liver diseases effect on liver cells integrity and it cuase an increase in serum activities of ALT and AST. Among of them, ALT is more specific biomarker for liver problems (Grigorescu, 2006; Kim et al., 2008; Nallagangula et al., 2017). Changes of AST serum activity happend keep less than ALT serum activity. In hepatic disorders Activities of ALT and AST may achieve as high as 100-times higher than normal average. But there is not strong connection between top level activities and prognoses may drop with aggravating of disease (Kim et al., 2008). In a case of AST/ALT proportion higher than 1 is a possibility of cirrhosis, with low specificity and high sensibility, and in chronic conditions this proportion is reach to 1 or less than it (Grigorescu, 2006; Kim et al., 2008; Nallagangula et al., 2017).

Alanine aminotransferase

Alanine aminotransferase (ALT) formerly known SGPT (serum glutamic-pyruvic transaminase), is an enzyme created by hepatocytes and When they are damaged, they released ALT into the bloodstream. ALT blood test is a type of liver function test which it blood serum levels can be elevated as a result of hepatocytes injury or death, by continuation of cell damaging, more liver cells produce ALT and the level of that keep increasing (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016;

Jarsiah et al., 2017; Hasan et al., 2018) (Table 4.). Drugs, chemical materials, bacteria poisons, viruses, and etc. may also be causes for an unusual increase in ALT serum levels (Table 3, 5.). High serum amounts of ALT in blood serum might demonstrate hepatic dysfunctions from liver inflammation, infection, cirrhosis, liver cancer and etc. (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). But, ALT is not reliable biomarker for detecting liver intact status by itself (Johansson et al., 2008; Ennulat et al., 2010; Kasarala and Tillmann, 2016; Jarsiah et al., 2017; Hasan et al., 2018). ALT serum levels can show fallen in the inflamed and fibrotic liver, hence just a liver biopsy can unclose the accurate status of the liver (Table 3, 5.). For this reason it is significant to have a diversity of tests to distinguish liver health status. Normal ALT levels are commonly reported as 10 to 40 IU/L (international units per litre) for rats (Ennulat et al., 2010; Hasan et al., 2018).

Aspartate aminotransferase

Formerly known SGOT (AST), is an enzyme similar to ALT but is less specific for hepatic dysfunctions. Due to many hepatic injuries, the ALT and AST levels are increased (Thapa and Walia, 2007). ALT is localized to the cytosol whilst AST is available in the cytosol and mitochondria of the liver cells (Rosen and Keeffe, 2000; Thapa and Walia, 2007). The normal range of AST in rats is between 50 to 150 IU/L (Hassan et al., 2018). The majority of the blood ALT activity is derived from the cytosolic isoenzyme, whilst majority activity of liver AST is derived by the mitochondrial isoenzyme (Rosen and Keeffe, 2000; Friedman et al., 2003; Thapa and Walia, 2007). High levels of serum AST by origin mitochondrial can occurs due to presence of necrosis in tissue like chronic hepatic dysfunctions or heart infarction (Nalpas et al., 1986; Rosen and Keeffe, 2000). ALT and AST amounts are elevated approximately in all hepatic disorders and severe blood serum increasing of ALT and AST can happen in a situation such as viral hepatitis and toxins or drugs which are brought about liver necrosis (Rosen and Keeffe, 2000; Friedman et al., 2003; Thapa and Walia, 2007) (Table 4.). Moderate blood serum increasing of ALT and AST (Such as acute liver disease, chronic liver disease, drug induced hepatitis, autoimmune hepatitis, and acute biliary tract blockage) Apart from chronic liver disease, the level of ALT is generally more often elevated as contrast to AST levels (Rosen and Keeffe, 2000; Friedman et al., 2003). Mild blood serum

increasing of ALT and AST (such as septic hepatitis, fatty liver, cirrhosis, drug toxicity, muscle inflammations and even after severe activities), however it worthy to keep in mind that sometimes in healthy liver a mild increasing in ALT could be happened (Katkov et al., 1991; Rosen and Keeffe, 2000; Thapa and Walia, 2007) (Table 3, 5.).

Alkaline phosphatase

The bile ducts and skeletal tissue are responsible for the production of the alkaline phosphatase (ALP) enzyme present in the liver (Burtis et al., 2012; Nallagangula et al., 2017). ALP Levels are increased in hepatitis, cirrhosis, other illnesses (Table 3, 5.) and some medications. Normal amounts of ALP in rats are between 44 to 147 IU/L (Burtis et al., 2012; Nallagangula et al., 2017). As mentioned earlier ALP amounts are elevated in liver dysfunctions, cirrhosis, and other disorders. Some drugs may also have knock on effect on this elevation. In alkaline ambient, ALP has catalyze action which responsible for hydrolyzing of phosphate ester. In any case of bile ducts disorders like any malfunctions or blockage of enzyme discharging bring about ALP incremental response in canalicular membrane of liver cells (Kim et al., 2008; Burtis et al., 2012; Nallagangula et al., 2017), besides that increase of ALP blood serum levels in extrahepatic blockage is more remarkable than intrahepatic blockage (occasionally 10 times upper than reference levels). About the connection of liver diseases and ALP activity, in a case of any dysfunctions in liver parenchymal cells like infectious hepatitis there might be normal to moderate elevation in ALP blood serum level (Burtis et al., 2012; Nallagangula et al., 2017).

Gamma-glutamyl transferase

The source of this enzyme is in bile ducts and it is clear that in the event of any disorder in bile ducts function, there are an elevation in its serum amounts (Table 3, 4.). Basal amounts of blood serum GGT in rats is down or undiscoverable (Leonard and Popp, 1984; Muthulingam et al., 2010). As a consequence of less normal serum levels and lack of related articles betwixt serum GGT and liver diseases, it has not been taken to consideration as a helpful biomarker of liver problems (Leonard and Popp, 1984; (Muthulingam et al., 2010). But regardless of cause, normal to Moderate elevations of GGT occurs in infectious hepatic dysfunction and injury (Wu et al., 1981; Moreira et al., 2017). Nevertheless, lots of surveys have shown that it is a biomarker of cholestasis and

biliary blockage (Wu et al., 1981; Moreira et al., 2017). Serum GGT and ALP are both quickly increased by bile duct disorders (Leonard and Popp, 1984).

Table 3. When Serum transferase levels may not reflect clinical hepatobiliary disease (Rochling, 2001; Woreta and Alqahtani, 2014)

The enzymes	Alteration
ALT	●Hepatocytes ●AST>ALT= drugs, cirrhosis ●Elevation of ALT and AST= ischaemic hepatitis, viral hepatitis, paracetamol
AST	●Hepatocytes, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lung, blood cells
ALP	●Biliary epithelium, bone, placenta, kidneys, gut
GGT	●Liver and other viscera ●Main role is to help interpret ALP ●ALP >> GGT= bonedisease, pregnancy ●GGT >> ALP= drugs, medications

Table 4. Typical biochemical features of certain hepatic disorders (Rochling, 2001; Woreta and Alqahtani, 2014)

Liver Diseases	Albumin	Bilirubin	ALT/AST	ALP	GGT
Acute alcoholic hepatitis	N	↑	↑	↑	↑↑
Acute viral hepatitis	N	↑↑	↑↑	↑	↑↑
Chronic viral hepatitis	↓or N	↑or N	↑	↑or N	↑
Cirrhosis	↓	↑or N	↑	↑	↑
Primary biliary cirrhosis	↓or N	↑↑	↑	↑↑	↑↑
Tumour secondaries	N	↑or N	↑	↑↑	↑↑

↑: raised, **N**: normal, ↓: reduced, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: γ-glutamyl transferase

Albumin

One of the important functions of the liver is protein metabolism which is cause producing of plasma protein, de-amination of amino acids, generating urea from ammonia and amino acid synthesis (Ericson and Sjöbäck, 1996). Albumin is a protein with liver origin which is flow in the blood. Decrease in blood serum albumin means liver dysfunction which can cause peripheral edema (cumulation of liquid on the far organ) and ascites (cumulation of liquid in the abdominal section) usually found in last stage hepatic disorders (Ericson and Sjöbäck, 1996; Burtis et al., 2012). Normal serum plasma albumin level is 30.8 g/l (Lillie et al., 1996). The amount of Albumin in blood serum is generally normal in chronic hepatic disorders until occurring of severe considerable progressing in hepatic disorder, In other words, the liver continues to produce albumin to the extent that half the parenchyma of the liver is lost. Plasma albumin is remarkable biomarker for detecting severity of the liver disease. But, its advantage for this object is limited, since plasma albumin levels is also reduced in renal failure (Lillie et al., 1996; Burtis et al., 2012).

It is possible to determine the cause of severe hypoalbuminemia from a combination of clinical findings, measurement of the serum globulin concentration, urinalysis (including protein creatinine ratio), tests of gastrointestinal protein loss, and tests of liver function. Decrease in blood serum albumin level might be indicated as liver disorders. But, decrease in blood serum albomin may be happened as a result of other problems beyond liver insufficiency such as severe exudative skin disease and protein-losing nephropathy (Lillie et al., 1996; Burtis et al., 2012).

To maintain serum albumin concentration, the amount of albumin synthesis should be equal to the amount of albumin loss. Slight reductions in blood serum albumin amount could happen as a result of different situations (Lillie et al., 1996; Burtis et al., 2012). As albumin contributes significantly to colloid oncotic pressure severe hypoalbuminemia can lead to ascites, pleural effusion, and/or subcutaneous edema. Albumin has a serum half-life of almost 36 hours in a fast growing rats, to 48 hours for a slower growing rats, to 60 hours for adult rats (Lillie et al., 1996; Burtis et al., 2012). Consequently, hypoalbuminemia isn't sensitive indicator for liver insufficiency and is more detected in portosystemic shunts (PSSs) and progressive chronic hepatic disorders (Lillie et al., 1996; Burtis et al., 2012).

Globulin

Globulins are not exclusively produced in the liver, that is to say α-globulins and β-globulins are produced in liver, whereas immunoglobulins (γ-globulins) originate from lymphoid cells. Hence Hepatic insufficiency rarely leads to a decrease in serum globulin concentration (Bauer et al., 1985; Jaleel and Nair, 2004)

Bilirubin

By the aging of red blood cells, cytochromes, and myoglobin their haemoglobin, which is consisted of "heme", converted to bilirubin (known as unconjugated bilirubin) via phagocytosis process in the liver, spleen and also in marrow. Albumin is responsible to carry unconjugated bilirubin to the liver cells (Bush, 1991; Yamaguchi et al., 1996; Berrahal et al., 2007). In the liver, bilirubin is conjugated via glucuronic acid then discharge to bile ducts (Fillit et al., 2016). In the duodenum some of the secreted bilirubin is absorbed and some of it is oxidized to stercobilinogen or turned to urobilinogen and finally some of it is eliminated as yellow urobilin via urination. In a case of liver injury, like acute liver inflammation or end stage liver disease, bilirubin cumulates in the blood and causes yellowing of the skin and eyes, known as jaundice (Bush, 1991; Fillit et al., 2016) (Table 5.).

Bilirubin amounts are generally stable until a severe amount of hepatic dysfunction has happened (Table 5.). Total bilirubin (normal value 4-7 μmol/L) (Yamaguchi et al., 1996; Berrahal et al., 2007; Houtmeyers et al., 2016), direct (the quantity of "conjugated" bilirubin or bilirubin that has been chemically connected to a glucuronide in the liver and then discharge from hepatocytes into the bile ducts and gathered in the gallbladder (absent in rat) or transferred to the duodenum then It moves to the intestines and being eliminated through faeces). And indirect (the amount of "unconjugated" bilirubin or free bilirubin that has not been attached to the glucuronide molecule). Consecutive evaluation of bilirubin is useful in recognise the severity of hepatic dysfunctions due to various ethology (Yamaguchi et al., 1996; Berrahal et al., 2007). Evaluation of blood serum bilirubin concentration can be applied along with other liver tests (Bush, 1991; Yamaguchi et al., 1996; Berrahal et al., 2007). Liver cells

totally remove and conjugate it with glucuronic acid by the UGT1A1, which is an enzyme in UGT groups (Yamaguchi et al., 1996; Berrahal et al., 2007).

Increase in unconjugated bilirubin can be increased as a result of effect some drugs on UGT1A1 function without anemia or hepatic disorders (Bush, 1991; Yamaguchi et al., 1996). This metabolite is refer to as unconjugated (indirect reaction) bilirubin. Hepatocytes efficiently remove it from the sinusoidal blood and conjugate it with glucuronic acid: a process that is catalyzed by UGT1A1 (Bush, 1991; Yamaguchi et al., 1996). Conjugated bilirubin is repelled into the bile and transported to the gut then transformed to urobilinogen and stercobilin by bacteria (It causes the stool to brown). In acute liver injury, serum bilirubin increases later than enzymes but stay high for longer time (Bush, 1991; Yamaguchi et al., 1996; Berrahal et al., 2007; Fillit et al., 2016). Liver hyperbilirubinemia may have seen in acute liver disorders or chronic liver diseases due to elevated in both unconjugated and conjugated bilirubin (Bush, 1991; Yamaguchi et al., 1996; Berrahal et al., 2007; Fillit et al., 2016). Evaluation of urobilinogen in urine is an old examination that was help to diagnose of bile duct patency, but todays, it is not worthy to assessment clinically (Bush, 1991; Yamaguchi et al., 1996; Berrahal et al., 2007).

Table 5. Typical patterns of clinicopathologic changes associated with liver disease (Sherlock and Dooley, 2002; Li et al., 2012).

Laboratory Test	Acute Hepatitis /Hepatic Necrosis	Chronic Hepatitis	Cirrhosis	Biliary Tract Obstruction	Non Obstructive Biliary Tract Disease	Hepatc Neoplsia
ALT	↑↑-↑↑↑	↑-↑↑↑	N-↑↑	N-↑↑	N-↑↑	N-↑↑
ALP	↑-↑↑	↑-↑↑	N-↑↑	↑↑↑	↑-↑↑↑	N-↑↑
Total bilirubin	N-↑↑↑	N-↑↑	N-↑↑↑	↑↑- ↑↑↑	N	N-↑
Preprandial SBA	N-↑↑	N-↑↑	↑-↑↑↑	↑↑-↑↑↑	N	N-↑
Postprandial SBA	N-↑↑	N-↑↑	↑-↑↑↑	↑↑-↑↑↑	N	N-↑
Ammonia	N-↑↑	N-↑↑	N-↑↑	N	N	N-↑

↑: Mild increase. ↑↑: moderate increase, ↑↑↑: severe increase, ALP: serum alkaline phosphatase activity; ALT: serum alanine aminotransferase activity; SBA: serum bile acid concentration.

A variety of bacterial infections can release harmful toxins that can effected canalicular membrane function, due to retention of conjugated bilirubin in the blood flow, but there is no physical biliary obstruction and it is thought a functional impairment of bile flow. Usually there is very little concomitant alter in liver enzyme acting's (Bush, 1991; Yamaguchi et al., 1996; Sherlock and Dooley, 2002; Berrahal et al., 2007; Li et al., 2012; Fillit et al., 2016). Increase in serum bilirubin levels could be the result of hepatobiliary or extrahepatic disorders and elevated in bilirubin can be resolves with successful management of the extrahepatic infection (Bush, 1991; Yamaguchi et al., 1996; Sherlock and Dooley, 2002; Berrahal et al., 2007; Li et al., 2012; Fillit et al., 2016). Prehepatic increase of bilirubin can happen as a result of bilirubin producing due to hemolysis (Sherlock and Dooley, 2002; Li et al., 2012).

A rapid devastation of erythrocyte because of intense hemolytic disorders as a result of raise in bilirubin is associated with anemia, which may cause an abnormality in liver enzyme functions. Both bile duct obstruction and hepatic disorders bring about an increase in bilirubin concentration and it is hard to separate them from each other. Ultrasound techniques and liver biopsy are applied to diagnose bile duct patency in both human medicine and veterinary medicine (Bush, 1991; Yamaguchi et al., 1996; Berrahal et al., 2007). Different concentrations of a second type of conjugated bilirubin could be created by prolonged cholestasis. There is a huge reserve capacity for bilirubin excretion in liver so, in order to cause hyperbilirubinemia by hemolysis, hepatic bilirubin clearance capacity must be decreased (Bush, 1991; Yamaguchi et al., 1996; Sherlock and Dooley, 2002; Berrahal et al., 2007; Li et al., 2012; Fillit et al., 2016). Hemolytic anemia due to hypoxia deficiency can lead to liver dysfunction. Increased SBA concentrations suggest hepatic dysfunction, PSS, or cholestasis, but they are not specific for any particular liver disease. High bilirubin levels leads to icterus. Conjugated bilirubin (biliprotein or δ-bilirubin) has bound with albumin (Bush, 1991; Yamaguchi et al., 1996; Sherlock and Dooley, 2002; Berrahal et al., 2007; Li et al., 2012; Fillit et al., 2016). Once, the cholestatic liver disease process is resolved, δ-bilirubin is removed from the circulation (Bush, 1991; Yamaguchi et al., 1996; Sherlock and Dooley, 2002; Berrahal et al., 2007; Li et al., 2012; Fillit et al., 2016).

Prothrombin time

Prothrombin time (PT), known as a blood clotting test, elevates when the blood levels of some blood clotting factors made by the liver decrease. In chronic hepatitis, the PT is generally not increased until cirrhosis is present and hepatic dysfunction is fairly remarkable. In severe liver injury, PT stays increased for a longer time (Grigorescu, 2006; Burtis et al., 2012; Nallagangula et al., 2017).

2.2.3. Hematological parameters in liver dysfunction

Complete Blood Counts (CBC) evaluate the three ingredients of blood: RBC, WBC, and platelets (PLT). White cell (leukocyte) count indicate the body’s ability to fight infection. While the body is actively fighting against an infection, the amount of total leukocytes increases, whereas when the amount of leukocytes decreases, it shows

a weak defence system against infection. Decrease in total leukocyte amount might be result in advanced hepatic malfunction or injury (Chung et al., 2016).

a) White blood cell count

White blood cell (WBC) counts like neutrophils, lymphocytes, monocytes, eosinophils, and basophils are always implemented in clinical examinations as indicates of systemic inflammation. Increased WBC counts have been related with several diseases like diabetes, cardiovascular problems, infection, metabolic diseases and liver disease (Chung et al., 2016).

Leukocytes (WBC) count gives detail on the body's capability to face infection. An increase in leukocytes count indicates the body is actively battling with infection; a decrease in leukocytes count indicates the low body's ability to battling with infection. Decrease in leukocytes count may be happened by advanced hepatic malfunctions or as effect of using some drugs (Chung et al., 2016).

b) Neutrophil count

Measurment of Neutrophil counts is implemented once there is an abnormality in normal body immune defence system against infections. A decrease in neutrophil count is known as neutropenia which also can happen as a result of Chemotherapy and treatment with interferon (Soza et al., 2002). On the contrary, an increase in neutrophil count (neutrophilia) together with Lymphocytopenia may indicate the intensity and progress of hepatic malfunctions (Ramaiah and Jaeschke, 2007; Von and Ley, 2008; Taylor et al., 2013; Xu et al., 2014; Chung et al., 2016). Neutrophils as main innate immune cell subset which participat in the first line of defence against infection, however they have a short life time. they are extremely important in the battle against any arousing inflammatory marks like tissue damage and microorganism attacking, these blood cells are presented in massive quantities in the bone marrow (Bonder et al 2004; Ramaiah and Jaeschke, 2007; Von and Ley, 2008; Taylor et al., 2013; Xu et al., 2014; Chung et al., 2016). The onset of liver damage can cause neutrophils infiltration as an acute reaction to new or persistent hepatic damage, liver stress or unknown systematic inflammatory marks. Neutrophils number generally elevated in intense hepatic injury. The

increased number of neutrophils beside lower lymphocyte count indicate as a reaction to injury or infection in patients (Ramaiah and Jaeschke, 2007; Von and Ley, 2008; Taylor et al., 2013; Xu et al., 2014).

c) Red cell count

The function of red cell count (RBC) is to deliver oxygen to cells and drive carbon dioxide away from them. Among all RBC parameters hemoglobin and hematocrit are the most significant value, and display interaction between oxygen and body cells (Gonzalez-Casas et al., 2009; Xie et al., 2016). Measurment of RBC count could manifest the level of liver injury, and also beside other tests may help to prediction the degree of liver dysfunction. It might be increased in a case of chronic liver dysfunction and decreased might be due to hepatic cirrhosis. RBC count indicate important differences between chronic liver dysfunction, hepatic cirrhosis, and hepatic cancer (Gonzalez-Casas et al., 2009; Xie et al., 2016). Anemia in liver diseases could happen as a result of gastrointestinal blood losing. Intense liver cells injury dispose to bleeding due to disorder in blood coagulation affected by shortage of blood coagulation factors produced by liver cells, and/or low levels of thrombocytes (Gonzalez-Casas et al., 2009; Xie et al., 2016).

d) Platelet count

It is referring to Provides information on the blood’s ability to clot. Thrombocytopenia, is a term used for indicate low platelet level in blood and is dangerous because of the risk of internal and external bleeding. The normal platelet count in rats is about 800 - 1000 $\times 10^3/\mu L$. Platelet production in the bone marrow is stimulated by thrombopoietin which is generated from liver so in a case of severe liver disease possibility of thrombocytopenia is high (Ishıkawa et al., 1998; Kurokawa and Ohkohchi, 2017). Advanced liver disease can cause thrombocytopenia which is the most common hematologic disorder developed along with severe liver disease cause poor prognosis, it often prevented critical interventions (Ishıkawa et al., 1998; Kurokawa and Ohkohchi, 2017).

Plateletcrit (PCT) is a measure of whole platelet valume in the blood which is a helpful screening way to detecting platelet abnormalcies. Mean platelet volume (MPV)

is average size of blood platelets and useful test for diagnose diseases of the bone marrow and bleeding problems. MPV affect on blood pct levels, which is bringing about overlap between normal platelets. Lots of studies have presented that mpv level could be increased due to liver dysfunctions. Therefore MPV level can reflect production of platlets in bone marrow (Purnak et al., 2013; Karagoz et al., 2014; Lida et al ., 2018)

e) Red blood cell distribution width

Red blood cell distribution width (RDW) is the rate of RBCs change in size and volume. An increase in RDW can happen due to RBCs enlargement. And it is generally occured on condition anemia, nutrient deficiency and also increased in liver disease. Increased RDW was observed in liver disease patients (Hu et al., 2013). RDW has direct relationship with creatinine levels and serum bilirubin, PT, and indirect relationship with serum albumin concentration and platelet counts. RDW is considered as an advance indication index of liver disorders (Hu et al., 2013).

2.2.4. Acute-phase proteins in liver dysfunction

Plasma proteins called acute phase proteins (APPs) which their concentration varies depending on the plasma concentration. Acute-phase proteins can be divided in two groups, it can be either positive acute-phase in a case of increasing (decrease albumin and instead increase other proteins such as serum amyloid A, haptoglobin, C-reactive protein, ceruloplasmin and alpha-1-acid glycoprotein), or else negative acute-phase proteins (such as albumin, retinol-binding protein, transthyretin, transferring and transcortin) (Cray et al., 2009). Both of them happen as a response to inflammation (Castell et al., 1989; Morgan et al., 1994). The acute phase refers to nonspecific inflammatory reaction of the animal that occurs shortly after any tissue injury (Castell et al., 1989; Morgan et al., 1994). The acute phase response and clinical application of monitoring APPs are diagnosed by a multiple of different systemic effects, such as fever, leukocytosis, elevated blood cortisol, reduction thyroxine concentrations, and metabolic alterations (Castell et al., 1989; Morgan et al., 1994).

2.2.5. Carbohydrate metabolism in liver dysfunction

The liver plays a central role in carbohydrate metabolism and is responsible conversion of galactose and fructose into glucose, gluconeogenesis, glycogen storage, and the synthesis of lots of compositions from carbohydrates (Serkova et al., 2006). Therefore in a case of liver problem there is failure in that passways and it brings about animals become susceptible for problems like diabetes mellitus. Liver disease in rats is determined by many metabolic changes, mainly catabolic, resulting in the clinical sign like malnutrition and even cachexia, lethargy (Nolte et al., 1995; Serkova et al., 2006). Blood pyruvate dehydrogenase in these animals beside total bilirubin and globulin levels were raised, but serum albumin level decreased significantly whilst concentrations of blood glucose, total lipids, total proteins, serum cholesterol and urea remained normal. Diabetes can occureas a result of a deficiency in carbohydrate metabolism in liver patients (Nolte et al., 1995; Serkova et al., 2006).

2.2.6. Plasma/Serum lipid in liver dysfunction

Lipid metabolism is one of the most important tasks of the liver, and also responsible for fatty acids oxidation, synthesis of lipoproteins, cholesterol production, and generates fatty acids by using carbohydrates and proteins (Muriel, 1998; Kundu et al., 2012). Hepatocytes produce primary bile acids from cholesterol which are secreted by the canalicular membrane into canaliculi and via bili ducts carry to the intestinal, after that chenodeoxycholic acid and cholic acid are conjugated with glycine or taurine. Conjugated bile acids are approximately totally absorbed (~95%) by the ileum into the portal blood flow and carried back to the liver for efficient first-pass absorb (70% to 80%) by hepatocytes that primarily located in periportal area (Muriel, 1998; Kundu et al., 2012). Conjugated bile acids are again excreted into the biliary system for another enterohepatic passway which they chip in the osmotic pressure that forces bile flow and creat surface-active detergent attributes that makes intestinal easily absorption of lipids (Muriel, 1998; Kundu et al., 2012).

Enterohepatic circulation system helps to recycling of bile acids. Small contents of initial bile acids not reabsorbed in the small intestinal are dehydroxylated by anaerobic bacteria in the colon to form secondary bile acids. Some of these are absorbed into the

portal venous flow and again recycled. Large amounts of bile acids in the portal circulation are composed of conjugated primary and secondary bile acids. Bile acids grasp by hepatocytes from the sinusoidal blood cover the sodium taurocholate cotransporting protein (NTCP) and the organic anion transporting protein (OATP), afterward bile acid carried into the bile ducts at the canalicular domain and then is moved by the bile salt export pump (BSEP) (Muriel, 1998; Kundu et al., 2012). In some species, like the rat, the gallbladder is absent and bile from the rat liver moves directly through the hepatic bile duct into the small intestine (the liver can produce bile on demand) (Fitzgerald, 1987; Behar, 2013). But in other animals after meal due to contraction of gallbladder, total bile acid concentrations increase in the circulation. Animal urine can be used to measure bile acids as needed (Sperry et al., 1940). To diagnose the liver pathology and designate its intensity Hepatic biopsy and microscopic survey is needed. Small intestinal bacterial overgrowth (SIBO) can increase the deconjugation of bile acids that are not effectively eliminated from the portal flow, which in turn may result in elevated circulating total bile acid. Hypoglycemia has a diagnostic value in liver disease which is often related to congenital portosystemic vascular problems and acute hepatic necrosis (Sperry et al., 1940). There is a connection between Hypoglycemia, glycogen storage inadequacy and initial hepatic neoplasia (Sperry et al., 1940). Liver-related low cholesterol levels is usually associated with connate portosystemic vascular disorders (Sperry et al., 1940).

Blood serum cholesterol amounts might be raised, normal, or declined in hepatic related disorders. Elevated or declined fasting serum cholesterol levels are not specific or sensitive for hepatobiliary problems. Serum triglyceride concentration may be raised or stay normal in patients with liver disease. Hypertriglyceridemia is related with raised blood serum liver enzyme levels (Sperry et al., 1940; Muriel, 1998; Kundu et al., 2012). However, an increased fasting serum triglyceride concentration is not a sensitive or specific marker for hepatobiliary disorders because they are also detected in patients with endocrine related problems, obesity, pancreatitis, and primary hyperlipidemia (Sperry et al., 1940; Muriel, 1998; Kundu et al., 2012).

2.2.7. Histopathologic analysis in liver dysfunction

One of the most important facets of the histopathological appraisal of liver biopsies in liver related problems is detecting of liver tissue structure change and its degree of fibrosis (Standish et al., 2006; Cheng and Wong, 2017). Clinician's index of suspicion for specific hepatic disorders could raise or decline due to laboratory test abnormalities, especially when expounded in association with the clinical findings, as well as the consequences of diagnostic imaging (Qi et al., 1999; Katsuyama et al., 2000; Standish et al., 2006; Cheng and Wong, 2017). Hence, Histopathological assessment of liver biopsies and sometimes recognition of a shunting blood vessel is usually needed to deterministic diagnose for hepatic disorders (Qi et al., 1999; Katsuyama et al., 2000).

2.2.8. Diagnostic imaging in liver diseases

Diagnostic imaging can play an important role to identify the presence of liver disorders, detect causes of secondary hepatopathy, as a way diagnosis specific hepatobiliary problem, and provide prognostic information. Radiography and abdominal ultrasound are the most frequently used imaging modalities for assessment of the hepatobiliary system in animals, but alternative imaging techniques are now being used more frequently (Bartolozzi, 1999; Murata et al., 2015). Nowadays, it seems clear that such diagnostic imaging will have a certain role in clinical practices. Such technique will progress detection and characterization of focal hepatic injuries (Bartolozzi, 1999; Murata et al., 2015).

2.2.9. Abdominal radiographs

Liver size, liver shape and opacity, and location can be assessed by abdominal radiographs (Bartolozzi, 1999; Murata et al., 2015). Radiography might as well help to diagnosing of extrahepatic disorders that affect the liver. But, it couldn't help to provide enough information's about hepatic parenchyma. Sometimes in patients with liver disorders abdominal radiographs couldn't help to diagnosis and it seems normal (Bartolozzi, 1999; Murata et al., 2015). One of the most practical advantages of using radiographic tecniques in liver related diseases is the measurement of liver size. Hepatic size, shape, opacity, and location in most patients may also allow identification of extrahepatic diseases that have effect on the liver Radiolucent areas in the liver indicate cumulation

of gas within the hepatic parenchyma, biliary tract, or portal vasculature (Bartolozzi, 1999; Murata et al., 2015).

2.2.10. Abdominal ultrasound

Ultrasound technique has been proved as a diagnostic approach for liver cirrhosis and fibrosis, however, few studies have been performed using this method to evaluate hepatic changes in rats (Chen et al., 1993; Guimond et al., 2007; Yan et al., 2007; Lessa et al., 2010). Abdomen Ultrasonography also is a technique which helps to recognizing diseases related to hepatic parenchyma and biliary tract. By the diagnosing hepatomegaly and microhepatia, secondary hepatopathy could also detecting as a result of extrahepatic disease (Guimond et al., 2007; Yan et al., 2007; De Graaf et al., 2008; Lessa et al., 2010). In order to make a definitive detection of liver related diseases, along with ultrasound technique, cytological and histological evaluation of liver tissue specimen, as well as blood hematology and biochemestery tests are generally indispensible (Guimond et al., 2007; Yan et al., 2007; De Graaf et al., 2008; Lessa et al., 2010).

2.2.11. Nuclear scintigraphy

Nuclear scintigraphy is a method of using a radioactive tracer (specially Technetium-99 m which is the most commonly used radiopharmaceutical) that is placed to a particular tissue (in this case liver) and it exposed to radioactive decay and then traced by a gamma camera and appears as an image (Bennink et al., 2005; De Graaf et al., 2008). Noninvasive tests of liver function in rats remains a challenge. Evaluation of local and total liver function for both uptake and excretion in bigger species is possible by using this technique. Nuclear scintigraphy generally used for specifying the portal circulation. By analyzing the radiation emitted from regions of liver, Transsplenic portal scintigraphy is highly specific and sensitive for the detect of congenital portosystemic shunt (Bioulac-Sage et al., 1985; Bennink et al., 2005; De Graaf et al., 2008). Studies in rats have shown that, Technetium-99 mebrofenin hepatobiliary scintigraphy (HBS) is noninvasive survey method which gives information and help to provide data by measuring based on quantities obtained of hepatic function (Bioulac-Sage et al., 1985; Malhi et al., 2002; Bennink et al., 2005; De Graaf et al., 2008).

2.2.12. Magnetic resonance imaging and computed tomography

Computed tomography (CT) and Magnetic resonance imaging (MRI) are fulfilled to discover liver parenchymal disorders (Qin et al., 1990; Yin et al., 2017). Accurate estimation of the liver mass is a significant achivement in the process of detecting liver diseases. Nowadays CT technique is fulfilled increasingly in human and animal medicine for the diagnosis of different liver vascular diseases. In human medicine MRI and CT techniques are used to detect liver parenchymal neoplasia (Qin et al., 1990; Yin et al., 2017). It has proved that both CT and MRI techniques have better accuracy compare to abdominal ultrasound and both of techniques have an improved exactitude for the recognition of liver neoplasia (Qin et al., 1990; Yin et al., 2017). But, there is not enough studies in the veterinary medicine field to evaluate their diagnostic efficiencies. Using of CT and sonography techniques for this purpose as a method of measuring liver mass are insufficient due to their measurement errors like as lack of spatial resolution (in a process of construction of a digital image) and inability of tissue differentiation (Qin et al., 1990; Yin et al., 2017). MR imaging is an exact technique to manifest the liver mass which is useful to measure small intrahepatic damages (Qin et al., 1990; Yin et al., 2017).

2.2.13. Vitamin D in liver dysfunction

There is a relation between the lack of vitamin D and prevalance of liver problems. Vitamin D is a fat-solvable sterol derivative that is mainly processed in the liver and has multiple functions (Roth et al., 2012; Keane et al., 2018). The deficiency and the lake of vitamin D is detect in several diseases related to chronic liver problems such as viral hepatitis B and C. Vitamin D receptors and Serum 25-hydroxyvitamin D are perhaps related with the outbreak, treatment and prognosis of diseases (Roth et al., 2012; Keane et al., 2018). One study showed that vitamin D deficiency in rats diet increased insulin resistance and toll-like receptor (TLR) activity as known a class of proteins that play a key role in the innate immune system (Roth et al., 2012; Keane et al., 2018). Vitamin D deficiency in rats brings about an increase in liver inflammations (Roth et al., 2012). Though the evidence of vitamin D supplementation in viral hepatitis and associated liver diseases is not enough, there is high potential to use vitamin D as a remedy to improving liver diseases. Though the main role of vitamin D is not truly

understood in liver relate problems, it is potentially beneficial in the treatment of chronic liver diseases. But, further investigations are still needed on this topic (Roth et al., 2012; Keane et al., 2018).

2.2.14. Kallistatin in liver diseases

Among of blood plasma proteins there is been a protein (kallistatin) which belongs to the serine protease inhibitor family (a tissue-kallikrein selective 427 amino acid 58-60 kD glycoprotein serpin). Kallistatin attaches firmly to tissue kallikrein however it's binding to other serine proteinases like chymotrypsin and elastase is weakly (Shen et al., 2008; Lin et al., 2013; Huang et al., 2014; Li et al., 2015).

It has been recently revealed that kallistatin is new and dependable biomarker for the diagnosis of hepatic diseases, due to close connection between the reduction in its serum amounts and development of early liver disease. Therefore kallistatin serum blood concentrations could help to detection of liver diseases as well as detect possible increase loss of liver function during therapy (Shen et al., 2008; Lin et al., 2013; Huang et al., 2014; Li et al., 2015). Kallistatin has known as modulator and vasodilator of vascular development, and it has anti-inflammatory, anti-oxidant and antiangiogenic affects. Kallistatin is present in a large among of fluids and tissues, including blood vessels, plasma, urine, liver, kidney and myocardium (Jenkins et al., 2010; Lin et al., 2013; Huang et al., 2014; Li et al., 2015). This serine protease inhibitor connects specifically to tissue kallikrein and inhibits its proteolytic effects. But, heparin can neutralized this inhibitory effect. The main place of kallistatin generate is the liver, and to a lesser extent the pancreas, lung, heart, kidney, large intestine, and other tissues. Kallistatin takes part in the anti-inflammatory reactions and cellular conformity against oxidative stress. As mentioned before kallistatin is present in the blood cells and endothelial cells and takes part in cardiovascular function (Chao et al., 1996; Huang et al., 2014; Li et al., 2015). Oxidative stress can cause a decrease in kallistatin serum level in blood vessels and kidney; reduction of endogenic kallistatin with anti-kallistatin antibody worsens renal and cardiovascular oxidative stress and result in inflammation (Huang et al., 2014; Li et al., 2015). This serine protease inhibitor (serpin) has specifically inhibits tissue kallikrein (Lin et al., 2013; Huang et al., 2014). This serine protease inhibitor (serpin) is known as a negative acute-phase protein, since its generation in the liver is quickly decreased after induction

inflammation by lipopolysaccharide (Shen et al., 2008). Kallistatin protecting organs and cells against inflammation, fibrosis, and oxidative stress, however, its connection to liver problems stays unclear (Shen et al., 2008; Cheng et al., 2015; Li et al., 2015). And yet the administration of kallistatin can diminish the development of liver disease, therefore the important aim of this study is to demonstrate change in the level of kallistatin in hepatic disorders in rat model. By meaguring rats blood serum kallistatin levels in a case of liver disorders and compared with healthy cases, a decrease in amounts could lead to finding out the severity of disease or might be help to early diagosis (Jenkins et al., 2010; Lin et al., 2013; Huang et al., 2014).

2.2.15. Diagnosis and assessment of liver fibrosis

Correct evaluation of the extent of liver injury is vital for clinical diagnosis whereby it is possible to predict prognosis and therapeutic decision in patients with liver injury. Liver biopsy (regardless of improvement of potential diagnostic experiments for half a century) is accepted as main method to grading liver diseases and gives effective guidance about diagnosis like necrosis, fibrosis, steatosis and inflammation (Grigorescu, 2006; Burtis et al., 2012; Nallagangula et al., 2017). There are some risk associated when liver biopsy is done, such as bleeding, pain, damage to biliary system, hypertension, and with seldom mortality (Grigorescu, 2006; Burtis et al., 2012; Castera, 2012; Nallagangula et al., 2017). And after liver biopsy along with positive aspects it has also negative aspects like highly invasive approaching, inadequate specimen quality and inadequate tissue size which make it inappropriate (Grigorescu, 2006; Burtis et al., 2012; Castera, 2012; Nallagangula et al., 2017). These risks of liver biopsy have given necessity for finding of noninvasive diagnostic method for liver diseases. An appropriate biomarker ought to be tissue specific, sensitive to portend active damage, readily achievable in peripheral organ and affordable (Grigorescu, 2006; Kim et al., 2008; Burtis et al., 2012; Castera, 2012; Nallagangula et al., 2017). Biomarkers superiority to liver biopsy are that there are evaluated in serum by minimal invasive method, easy feasibility and broad access as well (Grigorescu, 2006; Kim et al., 2008; Burtis et al., 2012; Castera, 2012; Orasan et al, 2016; Nallagangula et al., 2017).

3. MATERIALS AND METHODS

3.1. Material

3.1.1. Animal material

The animal material of the study; 32 Wister Albino male rats with live weight ranging between 200-400 gr were be used at Van Yüzüncü Yıl Üniversitesi Experimental Animal Production and Research Centre. Animal experiments were approved by Animal Ethics committee. The rats were cared for due to protocols approved by the Animal Care and Use Committee and they were housed in a temperature-controlled environment (20–22°C) and 75±2% relatively humidity with a normal light/dark cycle, and acclimatized for 7 days before the experiment.

3.1.2. Devices used

1. Biochemistry Device (BS-120 Vet-Mindray®)

2. Centrifuge Device (Rotofix32®-Hettich)

3. ELISA reader (ELISA reader®- DAS)

4. Veterinary Hemogram Device (Veterinary MS4-s-Melet Schloesing Laboratories in Van Yüzüncü Yıl University).

3.1.3. Used drugs

-Xylazine HCI %2 [Rompun (Bayer ®)],

-Ketamine HCI %10 [ketasol (Richter Pharma)]

-Carbon Tetrachloride %99 [Sigma-Aldrich]

3.1.4. Laboratory materials

-Injector (2 ml)

-Gauze

-Cage

3.2. Method

The rats were randomly divided into 4 groups and which each group consisted 8 rats, these groups are schematically shown in Table 6. All the groups were named as below:

Group 1: Control group consist 8 male rate weighting 200-400 gr. This group is healthy and were given normal standard pellet diet.

Group 2 (Mild group): This consisted 8 male rat which were injected subcutaneously with 2 ml/kg of 25% CCl_4 in paraffin oil (Sigma Co., Milan, Italy) twice a week for 4 weeks (Li et al., 2016; Huang et al., 2014).

Group 3 (Moderate group): This consisted 8 male rat and the same procedure of group 2 had been applied for this group (injected subcutaneously with 2 ml/kg of 25% CCl_4 in paraffin oil twice a week for 4 weeks). Then the procedure was continued with subcutaneously injection of 2 ml/kg of 50 % CCl_4 in paraffin oil for the 2 weeks (for 6 weeks in total) (Li et al., 2016; Huang et al., 2014).

Group 4 (Severe group): This group consisted of 8 male rats. CCl_4 was administered to this group in three stages and in three different doses for a total of 8 weeks. Firstly, rats were subcutaneously applied twice a week for 4 weeks with a 25% CCl4 solution dissolved in paraffin oil at a dose of 2 ml/kg. Secondly, 50% CCl4 solution dissolved in paraffin oil was applied subcutaneously twice a week for 2 weeks at a dose of 2 ml/kg. And finally, 62.5% CCl4 solution dissolved in paraffin oil was applied subcutaneously at a dose of 2 ml/kg twice a week for 2 weeks (Li et al., 2016; Huang et al., 2014).

Table 6. Animals and experimental designs.

Group	**Numbers**	**Drug administration**
Control	8	
Mild dose	8	2 ml/kg of 25% CCl_4 in paraffin oil (4 weeks)
Moderate dose	8	2 ml/kg of 25% (4 weeks)+50% (2 weeks) CCl_4 in paraffin oil (6 weeks)
Severe dose	8	2 ml/kg of 25%(4weeks)+50%(2 weeks) +62.5%(2 weeks) CCl_4 in paraffin oil (8 weeks)

3.3. Laboratory Examinations

At the end of the study, all rats were anesthetized with the administration of the combination of xylazine and ketamine (60 mg / kg (k) +7.5 mg / kg (x)), and then were sacrificed at the same day. For as for haematological and biochemical analysis Venous Blood samples were directly be obtained through Cardiac puncture and Posterior vena cava then Samples were kept at −20 °C for future examinations for biochemical examinations blood samples gathered into tubes and then centrifuged at 3000 g for 10 min's at 4°C for serum preparation.

For histopathology surveys, samples were cut off from the liver and washed immediately with an ice-cold phosphate buffer saline (PBS) to remove blood then they were stored in 10 % formaldehid.

3.3.1. Hematological analysis

Blood samples were taken from both Posterior Vena cava and direct heart puncture for analysis of hematological and biochemical parameters by 25 gauge needle. Anticoagulant tubes with 3 ml EDTA were used for hematological examinations and jelly biochemical serum tubes for biochemical examinations. Measurement of hematological parameters: Lymphocyte (LYM) and monocyte (MON), Neutrophil (NEU), Hematocrit value (Hct), hemoglobin concentration, leukocyte count, RBC mean corpuscular volume (MCV), mean cell hemoglobin (MCH), Mean Platelet Volume (MPV), plateletcrit (Pct), and mean corpuscular hemoglobin concentration (MCHC) ratios of blood samples taken from anticoagulant tubes were measured by Veterinary Hemogram Device (Veterinary MS4-s-Melet Schloesing Laboratories in France).

3.3.2. Biochemical analysis

Blood samples taken in antiquagulant-free tubes according to the method to be used in biochemical examinations will be centrifuged at 3000 rpm for 15 minutes and their serum will be collected. The serum obtained were stored at -20 °C for doing measurements. ELISA (enzyme-linked immunosorbent assay) was done by using a polyclonal antibody to rat kallistatin (Chao et al. 1996). ELIZA techniques was implemented for analyzing of rat serum Kallistatin levels and measured by a mercantile

ELISA kit (Ylbiont) (catalog NO: YLA1624RA) due to the producer's instructions. As well Rat COL-I (Ylbiont) (catalog NO: YLA0195RA) and Rat COL-III (Ylbiont) (catalog NO: YLA0605RA) was measured due to the producer's instructions (Zhou et al. 1992; Chao et al. 1996; Li et al., 2015). In short, kallistatin sample and standards were added, in duplicate, into a 96-well microtiter plate previously coated with none labeled anti kallistatin IgG. Then all plates had incubated at 37°C for 90 min then washing process had performed and then was followed by adding biotin labeled anti kallistatin IgG, the plate was incubated at 37°C for 60 min. Before substrate addition, Peroxidase avidin was added and incubated at 37°C for 60 min. The plate was read at 414 nm using an ELISA reader, After a 30 min colour reaction (Zhou et al. 1992). Same technique were applied for both COL-I and COL-III. All levels were measured using commercial ELISA test kits according to their instructions.

Measurement of aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) levels, the blood serum was measured by commercial kits using autoanalyzer (BS-120 Vet-Mindray).

3.4. Histopathological analysis

Rats were humanely sacrificed at the end of experiment, and fragments from liver tissues were fixed in 10 % neutralized formaldehyde, embedded in paraffin and then, stained with hematoxylin and eosin, and histological specimens were examined via light microscopy.

3.5. Statistical analysis

Descriptive statistics were used for the properties of biochemical parameters of rat with liver damage and the healthy ones; Mean were expressed as Standard Deviation, Minimum and Maximum. The Biochemical parameters were compared between groups by Kruskal-Wallis test. Duncan multiple comparison test were used to Identifying different groups. To determine the relationship between these variables, Spearman correlation coefficients were calculated separately. Statistical significance levels were taken as 5% in the calculations and SPSS statistical package program were used for the calculations.

4. RESULTS

4.1. Clinical Findings

From observing the rats in group 1 (mild group n=8) was noted that 4 weeks after the first drug applying, the animals all were alive, and by the time passing by some of them began to manifest of mild weakness, somehow fatigue and in some animal changing in eye colour had detected. In group 2 all animals (moderate group n=8) after 6 weeks of first drug administration were alive but with signs like Poor coordination, frequent urination, somehow bloody diarrhea, face ulcers and some of them shown some degree of restlessness and poor physical activities. In group 3 (severe group n=8) at the end of drug administration 2 of the animals had dead and the rest of them shown sign of severe fatigue, significant decrease in food consumption, bloody diarrhea, severe ulcers around mouth and eyes, slow and deep breathing, Ascites (fluid in the abdomen), and severe degree of weakness and inactivity for most of hours of day presented (almost about to die) had noticed.

4.2. Laboratory Findings

Hematologic erythrocyte parameters (RBC, MCV, MCH, MCHC, Hct, Hb, WBC, NEU, LYM, MON, MPV, Pct levels) were determined from blood samples taken from tubes stained with anticoagulant tubes.

4.2.1. Hematologic findings

WBC, Neu, LYM, Mon, PCT levels of the rats belonging to liver damage groups were significantly higher than the control group ($p<0.05$), while MCH and MCHC levels were lower ($p<0.05$). Hemoglobin (Hb) and mean corpuscular volume (MCV) values were both showed significant difference between severe group and other groups ($p<0.05$). By comparison within the groups exposed to liver injury and control group, there was a significant difference between control group and mild and severe groups in hematocrit (Hct) value ($p<0.05$). There wasn't important different between control group and moderate group as well between mild group and severe group ($p>0.05$). But, although

RBC and MPV values were found to be mathematically high, they were not statistically significant ($p<0.05$) (Table 7.).

Table 7. Hematological parameters in control and liver damage of rat

Parametre	Control Group 1 (n=8) x̄±SD	Mild Liver Disorder Group 2 (n=8) x̄±SD	Moderate Liver Disorder Group 3 (n=8) x̄±SD	Severe Liver Disorder Group 4 (n=8) x̄±SD
WBC ($10^3/mm^3$)	$5.26±1.8^A$	$13.48 ±4.01^B$	$22.10 ±11.07^B$	$7.20 ±2.71^C$
RBC ($10^6/mm^3$)	$6.78 ±0.47^A$	$7.87 ±0.74^A$	$7.84 ±1.15^A$	$6.35 ±1.62^A$
NEU ($10^3/mm^3$)	$2.57 ±0.91^A$	$9.12 ±4.96^B$	$15.82 ±6.52^B$	$4.44 ±2.02^C$
LYM ($10^3/mm^3$)	$2.35 ±0.85^A$	$3.59 ±1.84^A$	$5.78 ±6.64^B$	$2.25 ±0.73^A$
MON($10^3/mm^3$)	$0.26 ±0.10^B$	$0.74 ±0.23^B$	$0.47±0.14^B$	$0.28 ±0.14^A$
Hct (%)	$39.04 ±2.54^A$	$43.80 ±3.81^B$	$40.94 ±3.49^A$	$35.81±9.35^B$
Hb (g/dl)	$16.74 ±0.53^A$	$17.55 ±1.75^A$	$15.23 ±1.38^A$	$12.93±3.70^C$
MCV (fl)	$57.65 ±1.41^A$	$55.60 ±1.18^A$	$55.55 ±3.22^A$	$52.84 ±6.09^C$
MCH (fl)	$24.71 ±1.48^A$	$22.21 ±.94^B$	$20.70 ±1.06^B$	$18.66±2.30^C$
MPV(fl)	$6.10±0.25^A$	$6.45 ±0.35^A$	$7.21 ±0.90^A$	$7.00 ±0.50^A$
MCHC (g/dl)	$43.05 ±2.19^A$	$40.03 ±2.00^A$	$37.16 ±1.66^C$	$35.41 ±2.43^C$
PCT (%)	$0.31 ±0.05^A$	$0.30 ±0.14^A$	$0.52 ±0.30^B$	$0.50 ±0.20^B$

A, B, C: Different lower cases in the same column represent statistically significant differences AB, AC, BC: $p<0.05$ and same lower cases in the same column represent statistically not significant differences. AA, BB and CC: $p>0.05$

4.2.2. Biochemical findings

In the statistical analysis of biochemical parameters, in the analysis of serum ALT levels; Serum ALT levels in rats with severe liver injury were significantly higher than those of control group and rats with mild and moderate liver injury (A, B). But, ALT level was not show any significant difference between rats belonging to control, mild and moderate liver injury groups (Figure 3) (Table 8, 9). In the analysis of serum AST levels; Serum AST levels of rats with severe liver injury were significantly higher than control group and rats belonging to mild and moderate liver injury. However, the difference between AST levels of control group and rats with mild liver injury was not significant (Figure 4) (Table 8, 10.). In the statistical analysis of biochemical parameters, in the analysis of serum GGT levels; Serum GGT levels in rats with severe liver injury

were significantly higher than those of control group and rats with mild and moderate liver injury (A, B). But, GGT level was not show any significant difference between rats belonging to control, mild and moderate liver injury groups (Figure 5) (Table 8, 11.). In the analysis of serum LDH levels; serum LDH levels of rats with severe liver injury were significantly higher than control group and rats with mild and moderate liver injury. Similarly, LDH levels in rats belonging to mild and moderate liver injury group were higher than control group, however there was no meaningful difference between the rats belonging to mild liver injury group and rats in moderate liver injury group (Figure 6) (Table 8, 12.).

In the analysis of serum Coll-I levels; Serum Coll-I levels of rats belonging to liver injury groups were significantly higher than control group. Similarly, serum Coll-III of rats belonging to liver injury groups were significantly higher than those control group (Figure 7, 8) (Table 8, 13, 14.).

In the analysis of kallistatin levels; kallistatin levels were lower in all groups with liver damage compared to the control group. Comparison between groups with liver injury showed that although there was a statistically significant difference between Kallistatin levels in the mild group and severe group, but there was no significant difference between mild group and moderate group as well as moderate and severe groups (Figure 9) (Table 8, 15.).

Table 8. Biochemical parameters in control and rat with liver injury

Parameter	Control Group 1 (n=8) x̄±SD (Min- Max)	Rat with liver injury		
		Mild liver injury Group 2 (n=8) x̄±SD (Min- Max)	Moderate liver injury Group3 (n=8) x̄±SD (Min- Max)	Severe liver injury Group 4 (n=8) x̄±SD (Min- Max)
ALT(IU/L)	30.81±4.26^{B} (26.30-36.20)	104.93±45.63^{B} (45.50-164.20)	261.63±72.19^{B} (175.20-354.10)	1076.46+±864.43^{A} (402.80-2558.30)
AST(IU/L)	96.07±33.28^{C} (29.40-127.40)	213.67±75.10^{C} (127.70-343.40)	448.81±84.04^{B} (357.10-587.20)	968.86±218.11^{A} (716.90-1367.40)
LDH(IU/L)	414.12±181.36^{C} (170.00-643.00)	804.50±82.12^{B} (676.00-897.00)	1077.25±191.97^{B} (892.00-1346.00)	2090.88±679.73^{A} (1438.00-3212.00)
GGT(IU/L)	1.37±0.51^{B} (1.00-2.00)	2.00±0.75^{B} (1.00-3.00)	2.12±0.99^{B} (1.00-4.00)	4.00±2.07^{A} (2.00-8.00)
Coll-I(ng/l)	5.97±1.77^{D} (4.26-7.96)	9.36±0.35^{C} (8.77-9.78)	10.75±0.68^{B} (10.10-11.98)	13.33±0.78^{A} (12.36-14.56)
Coll-III(ng/l)	218.16±31.48^{D} (177.27-277.98)	339.31±18.54^{C} (300.74-361.01)	380.89±17.99^{B} (364.27-410.54)	488.84±30.44^{A} (446.04-339.68)
Kal(ng/ml)	7.96±1.76^{A} (6.76-11.65)	6.48±0.18^{B} (6.23-6.74)	5.91±.024 ^{B,C} (5.58-6.23)	5.12±0.01^{C} (5.11-5.15)

Different letters (A, B, C, D) on the same line that indicate data are statistically significant. ($p<0.01$).

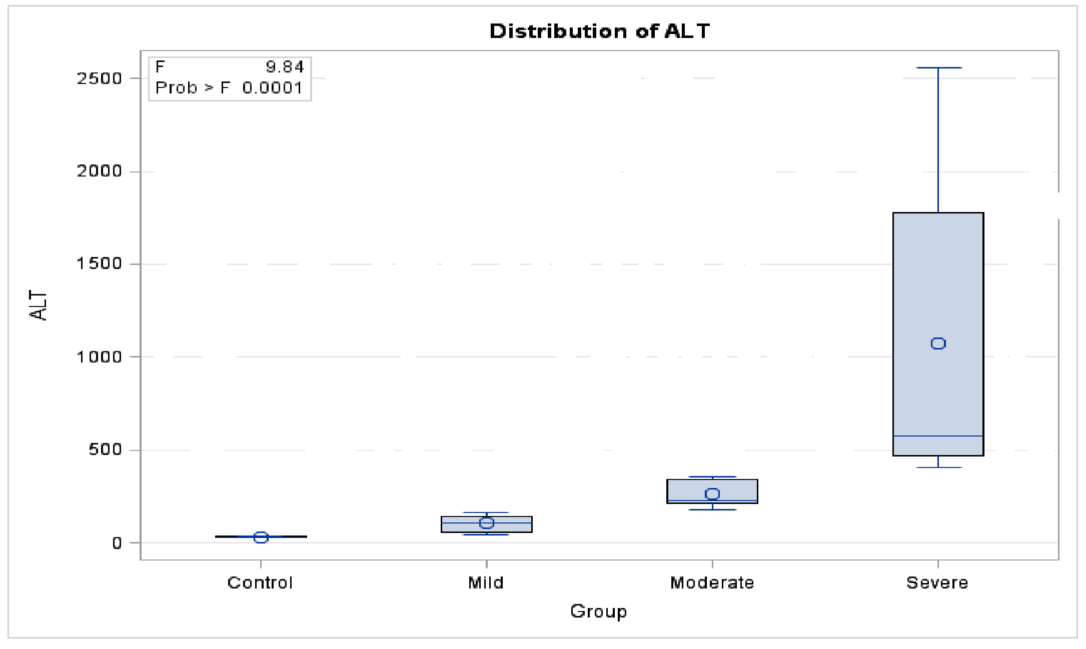

Figure 3. Serum ALT in control and rat with liver injury

Table 9. Explanation for ALT correlation in control and rat with liver injury

Means with the same letter are not significantly different			
Duncan Grouping	**Mean**	**N**	**Group**
A	1076.5	8	Severe
B	261.6	8	Moderate
B	104.9	8	Mild
B	30.8	8	Control

There is a meaningful difference between severe group and other groups (A, B), while there is no difference between control, mild and moderate groups (Figure 3 and Table 9.).

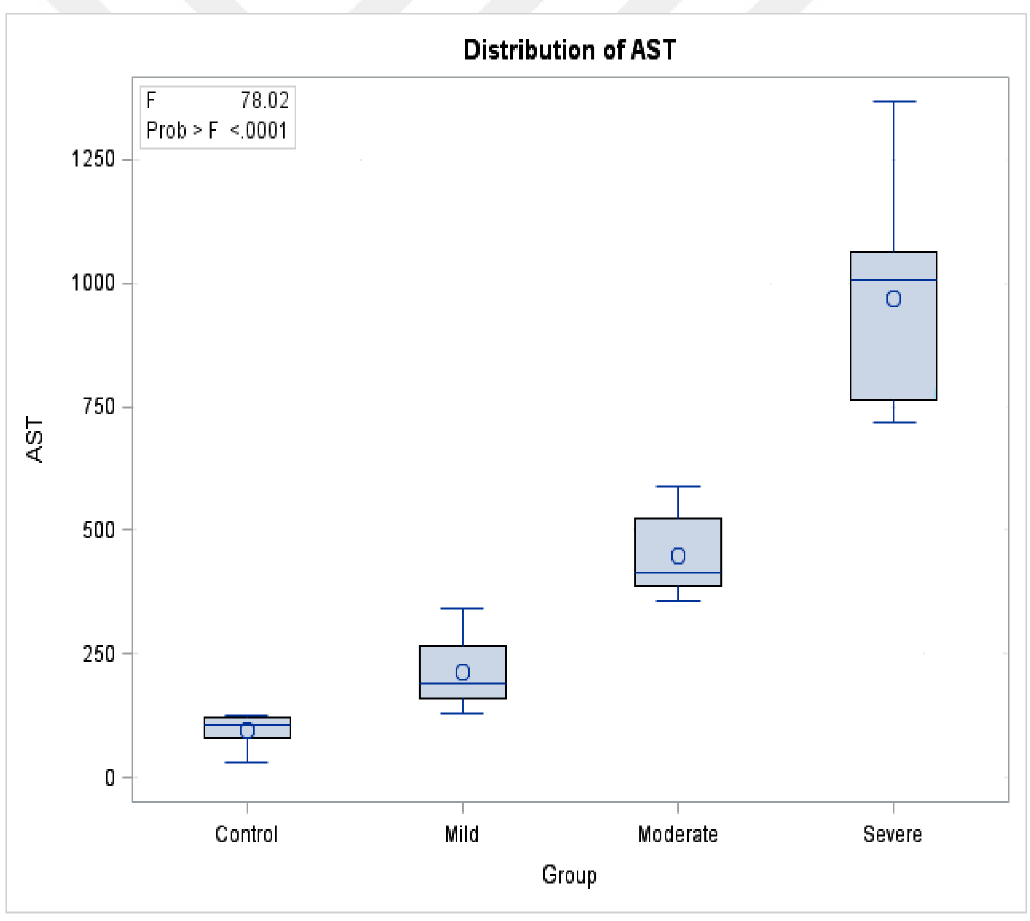

Figure 4. Serum AST in control and rat with liver injury

Table 10. Explanation for AST correlation in control and rat with liver injury

Means with the same letter not significantly different			
Duncan Grouping	**Mean**	**N**	**Group**
A	968.86	8	Severe
B	448.81	8	Moderate
C	213.68	8	Mild
C	96.08	8	Control

There is a meaningful difference between severe group and other groups (A, B, C), and also between mild and moderate groups (B, C), while there is no difference between mild and control groups (C) (Figure 4 and Table 10.).

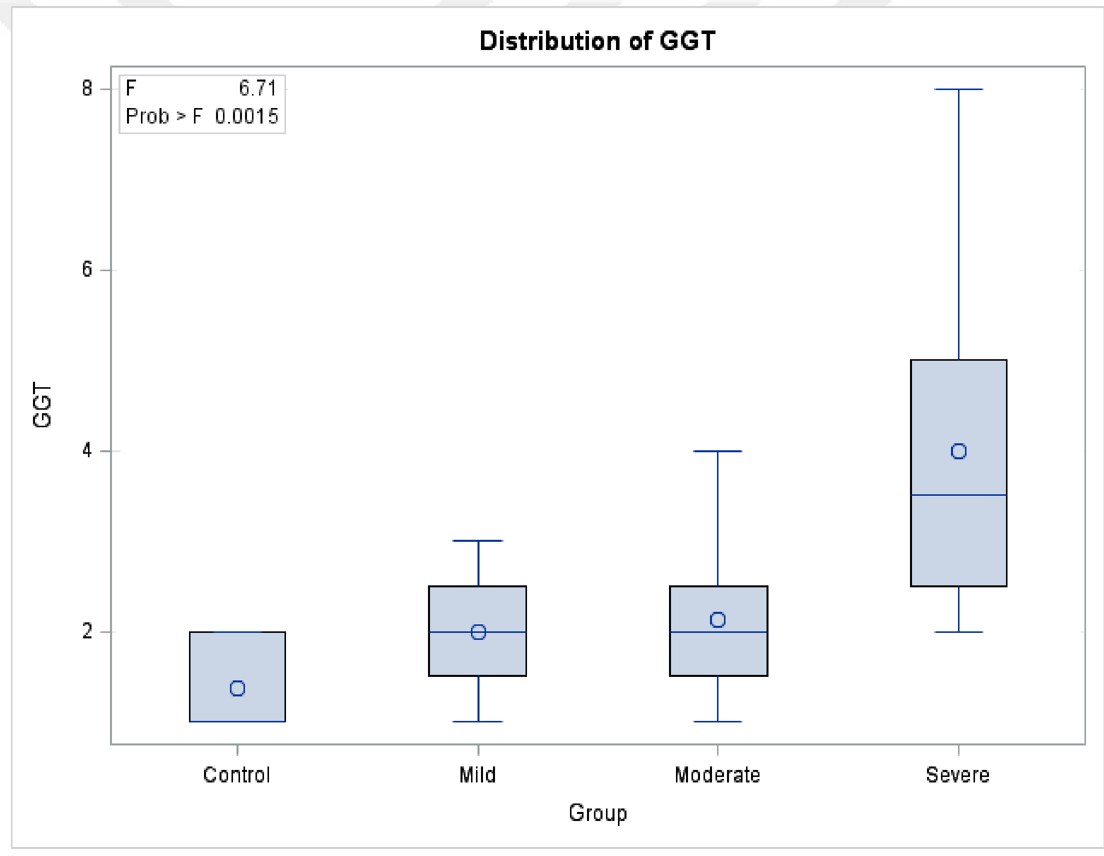

Figure 5. Serum GGT in control and rat with liver injury

Table 11. Explanation for GGT correlation in control and rat with liver injury

Means with the same letter are not significantly different			
Duncan Grouping	**Mean**	**N**	**Group**
A	4.0000	8	Severe
B	2.1250	8	Moderate
B	2.0000	8	Mild
B	1.3750	8	Control

There is a meaningful difference between severe group and other groups (A, B), while there is no difference between mild, moderate and control groups (B) (Figure 5 and Table 11.).

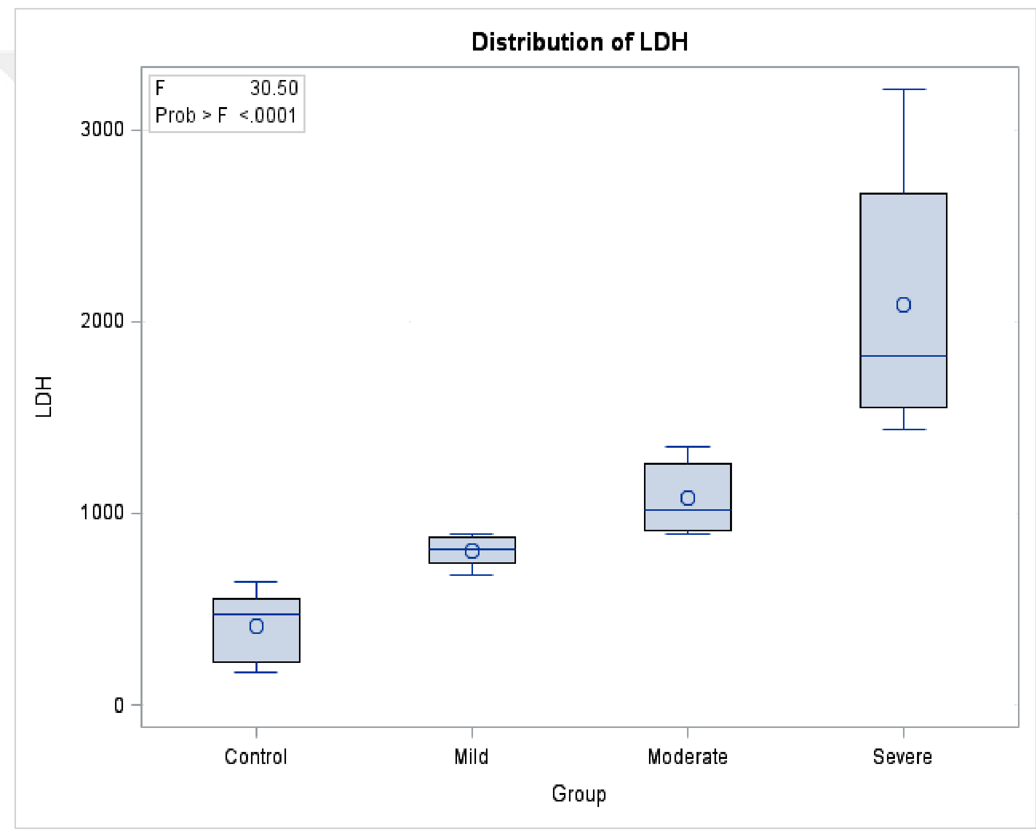

Figure 6. Serum LDH in control and rat with liver injury

Table 12. Explanation for LDH correlation in control and rat with liver injury

Means with the same letter are not significantly different			
Duncan Grouping	**Mean**	**N**	**Group**
A	2090.9	8	Severe
B	1077.3	8	Moderate
B	804.5	8	Mild
C	414.1	8	Control

There is a meaningful difference between severe group and other groups (A, B, C), and also between mild and control groups (B, C) as well between moderate and control groups (B, C). There is no difference between mild and moderate groups (B) (Figure 6 and Table 12.).

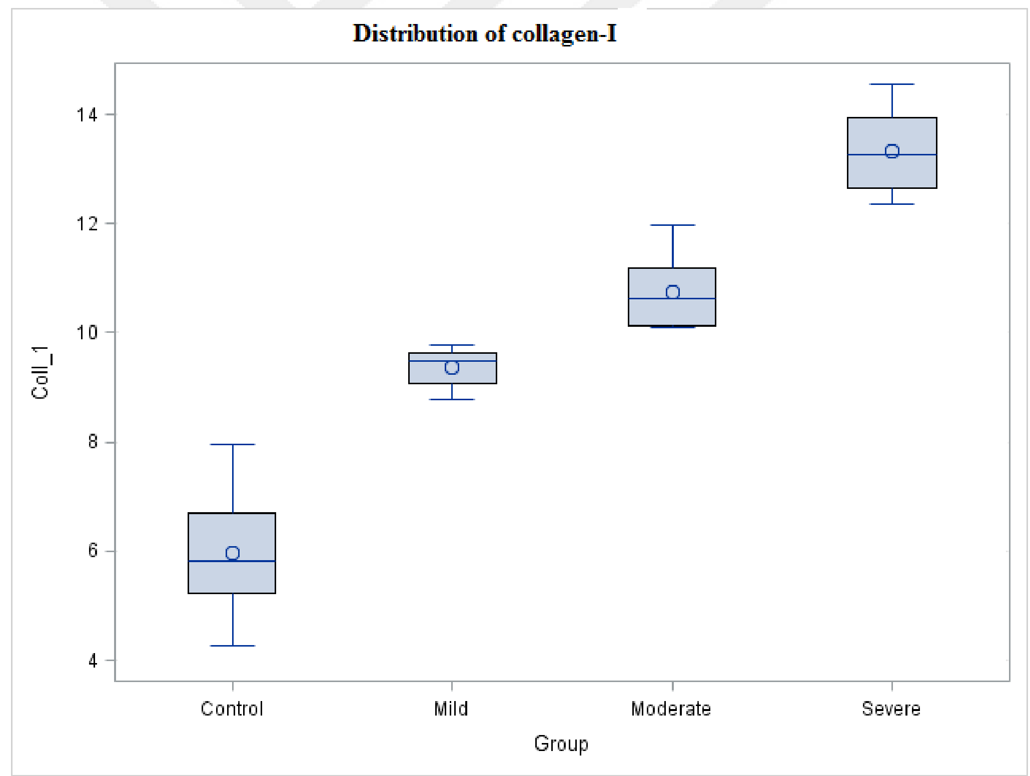

Figure 7. Serum Collagen type I in control and rat with liver injury

Table 13. Explanation for Col-I correlation in control and rat with liver injury

Means with the same letter are not significantly different			
Duncan Grouping	**Mean**	**N**	**Group**
A	13.3375	8	Severe
B	10.7525	8	Moderate
C	9.3688	8	Mild
D	5.9700	8	Control

There is a meaningful difference between control group and groups belonging to liver injury (D, B, C, A), and also between groups belonging to liver injury (A, B, C) (Figure 7 and Table 13.).

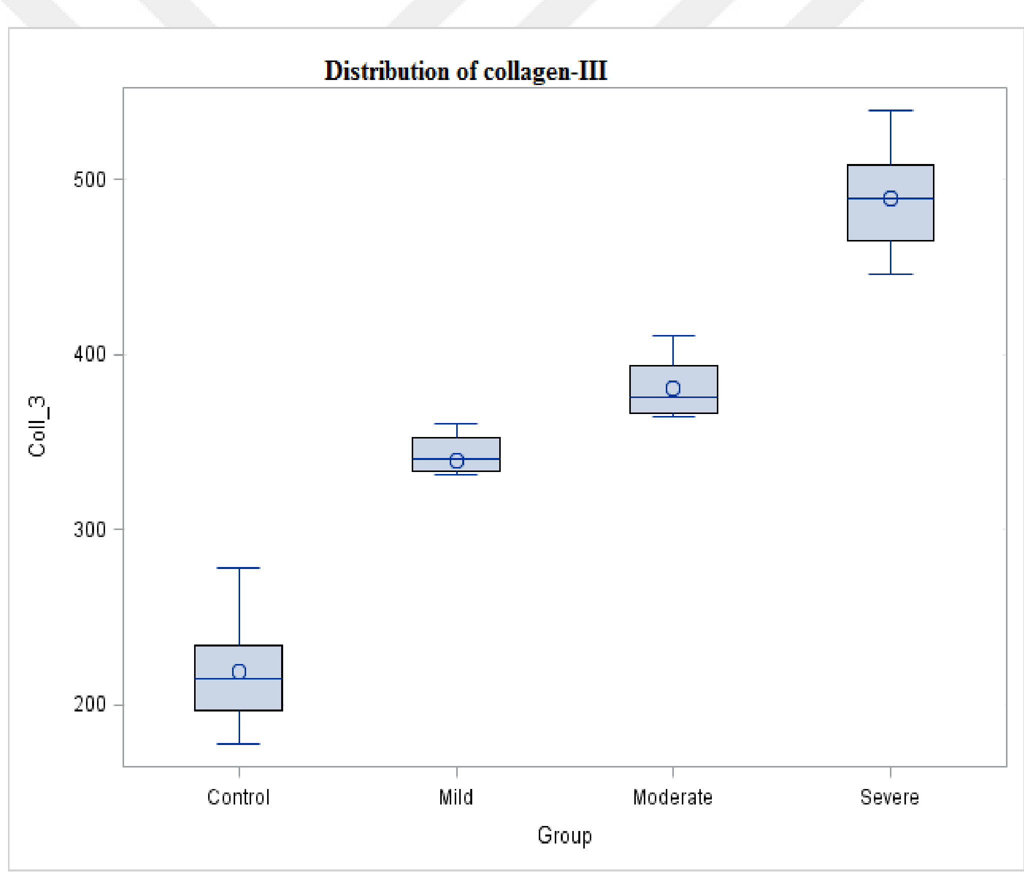

Figure 8. Collagen type III in control and rat with liver injury

Table 14. Explanation for Col-III correlation in control and rat with liver injury

Means with the same letter are not significantly different			
Duncan Grouping	**Mean**	**N**	**Group**
A	488.85	8	Severe
B	380.90	8	Moderate
C	339.32	8	Mild
D	218.17	8	Control

There is a meaningful difference between control group and groups belonging to liver injury (D, B, C, A), and also between groups belonging to liver injury (A, B, C) (Figure 5 and Table 14.).

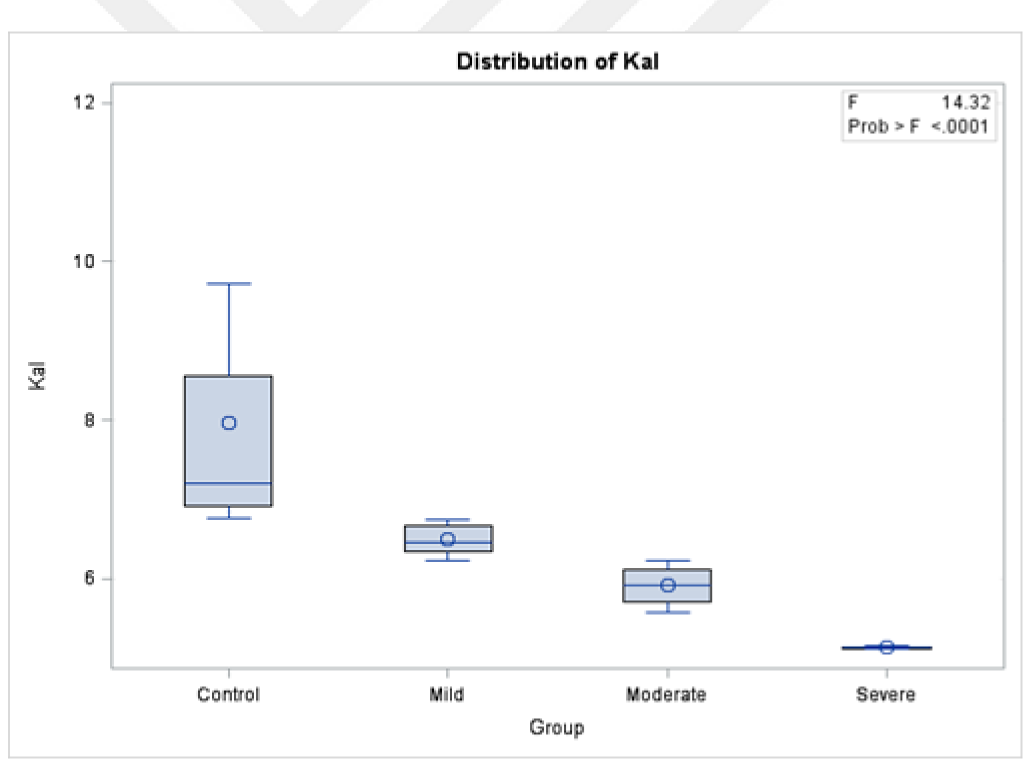

Figure 9. Kallistatin in control and rat with liver injury

Table 15. Explanation for kallistatin in control and rat with liver injury

Means with the same letter are not significantly different				
Duncan Grouping		**Mean**	**N**	**Group**
	A	7.9663	8	Control
	B	6.4863	8	Mild
C	B	5.9100	8	Moderate
C		5.1275	8	Severe

There is a meaningful difference between control group and groups belonging to liver injury (A, B, C), and also there is a difference between mild group and severe group (B, C) but there is no defference between moderate group and severe group (BC)as well mild group and moderate group (BC) (Figure 9 and Table 15.).

Table 16. The correlation between parameters in control group

	Kal	**Coll- III**	**Coll-1**	**ALT**	**AST**	**GGT**	**LDH**
Kal	1.00000						
Coll- III	-0.70662*	1.00000					
Coll-1	-0.75686*	0.93044***	1.00000				
ALT	-0.69498*	0.90376**	0.91866**	1.00000			
AST	-0.96371***	0.80300*	0.88471**	0.83557**	1.00000		
GGT	0.35173	-0.38570	-0.57447	-0.51387	-0.46376	1.00000	
LDH	-0.63929*	0.63794*	0.67922*	0.44189	0.65663*	-0.05080	1.00000

******* p<0.001, ****** p<0.01, ***** p<0.5

Intra-group analysis revealed that, there is a negative association between kallistatin levels and other biochemical parameters. That is to say with the reduction of kallistatin serum levels, the other biochemical parameter serum levels increasing (Table 16.).

Table 17. The correlation between parameters in mild group

	Kal	**Coll- III**	**Coll-1**	**ALT**	**AST**	**GGT**	**LDH**
Kal	1.00000						
Coll- III	-0.89713**	1.00000					
Coll-1	-0.96302***	0.92898**	1.00000				
ALT	-0.99016***	0.88420**	0.97027***	1.00000			
AST	-0.91724**	0.86083**	0.87668**	0.92234**	1.00000		
GGT	-0.23378*	0.12007*	0.25219	0.26792*	0.19500*	1.00000	
LDH	-0.97311***	0.92758**	0.98006***	0.98749***	0.89690**	0.24852*	1.0000

******* p<0.001, ****** p<0.01, ***** p<0.5

Intra-group analysis revealed that, there is a negative association between kallistatin

levels and other biochemical parameters. That is to say with the reduction of kallistatin serum levels, the other biochemical parameter serum levels increasing (Figure 9 and Table 17.).

Table 18. The correlation between parameters in moderate group

	Kal	Coll- III	Coll-1	ALT	AST	GGT	LDH
Kal	1.00000						
Coll- III	-0.92366**	1.00000					
Coll-1	-0.93953**	0.92704**	1.00000				
ALT	-0.91479**	0.87959**	0.90905**	1.00000			
AST	-0.92872**	0.91891**	0.95992**	0.98089***	1.00000		
GGT	-0.27345*	0.16197*	0.20934	0.22595*	0.23460*	1.00000	
LDH	-0.97037***	0.93188**	0.95626**	0.94898**	0.95769**	0.23709*	1.00000

******* p<0.001, ****** p<0.01, ***** p<0.5

Intra-group analysis revealed that, there is a negative association between kallistatin levels and other biochemical parameters. That is to say with the reduction of kallistatin serum levels, the other biochemical parameter serum levels increasing (Figure 9 and Table 18.).

Table 19. The correlation between parameters in severe group

	Kal	Coll- III	Coll-1	ALT	AST	GGT	LDH
Kal	1.00000						
Coll- III	0.06322*	1.00000					
Coll-1	0.10027*	0.98795***	1.00000				
ALT	0.30962	0.90823**	0.93450**	1.00000			
AST	0.10050*	0.97176***	0.95212**	0.84723**	1.00000		
GGT	0.23187*	0.74210*	0.78187*	0.86351**	0.75236*	1.00000	
LDH	0.18117*	0.90665**	0.95237**	0.96235***	0.82281*	0.77957*	1.00000

******* p<0.001, ****** p<0.01, ***** p<0.5

Intra-group analysis revealed that, there is a positive association between kallistatin levels and other biochemical parameters. That is to say by reducing of kallistatin serum levels, the other biochemical parameter serum levels also be reduced (Figure 9 and Table 19.).

Table 20. The correlation between parameters in control and rat with liver injury

	Kal	Coll- III	Coll-1	ALT	AST	GGT	LDH
Kal	1.00000						
Coll- III	-0.82420***	1.00000					
Coll-1	-0.85156***	0.99300***	1.00000				
ALT	-0.45746**	0.71013***	0.68750***	1.00000			
AST	-0.69376***	0.92133***	0.90308***	0.84421***	1.00000		
GGT	-0.41298*	0.65014***	0.62672***	0.84617***	0.74590***	1.00000	
LDH	-0.68131***	0.89841***	0.88650***	0.92225***	0.94980***	0.79588***	1.00000

*** $p<0.001$, ** $p<0.01$, * $p<0.5$

Intergroup analysis revealed that, there is a negative association between kallistatin levels and other biochemical parameters. That is to say with the reduction of kallistatin serum levels, the other biochemical parameter serum levels increasing (Figure 9 and Table 20.).

4.3. Histopathological findings

In the histopathology examination of liver tissue; there was no histological change in the livers of the control rat. Our results showed that bile duct, parenchymal cells and Kuppfer cells were normal. Hepatocyte necrosis, inflammatory cell infiltration and bleeding foci were detected in CCl_4 -induced groups. Histopathological examination of the liver in rats in the liver injury groups and control group revealed normal histological appearance in the liver tissue. But, severe morphological changes were detected in the liver tissue of rats belonging to groups of liver damage (mild, moderate and severe) caused by carbontetra chloride. These findings were scored in table 21, and hydropic degeneration and clotting necrosis was detected in hepatocytes especially in the vena centralis and mid-zone regions. In some of vena centralis (Periacinary fibrosis) and portal areas Fibrosis was present. It was only observed the spread of thin collagen bundles from the portal marks to the parenchyma. It was also observed activation in perisinusoidal myofibroblastic cells. Inside the the portal tracts and liver parenchyma, lymphoid cells were time to time observed that were contains some macrophages. These changes resulted in cell decomposition, and also disruption of liver lobe architecture in the liver parenchyma in severe group which were induced by CCl_4. In the study we found that in control group histological appearance of the liver was normal (Figure 10).

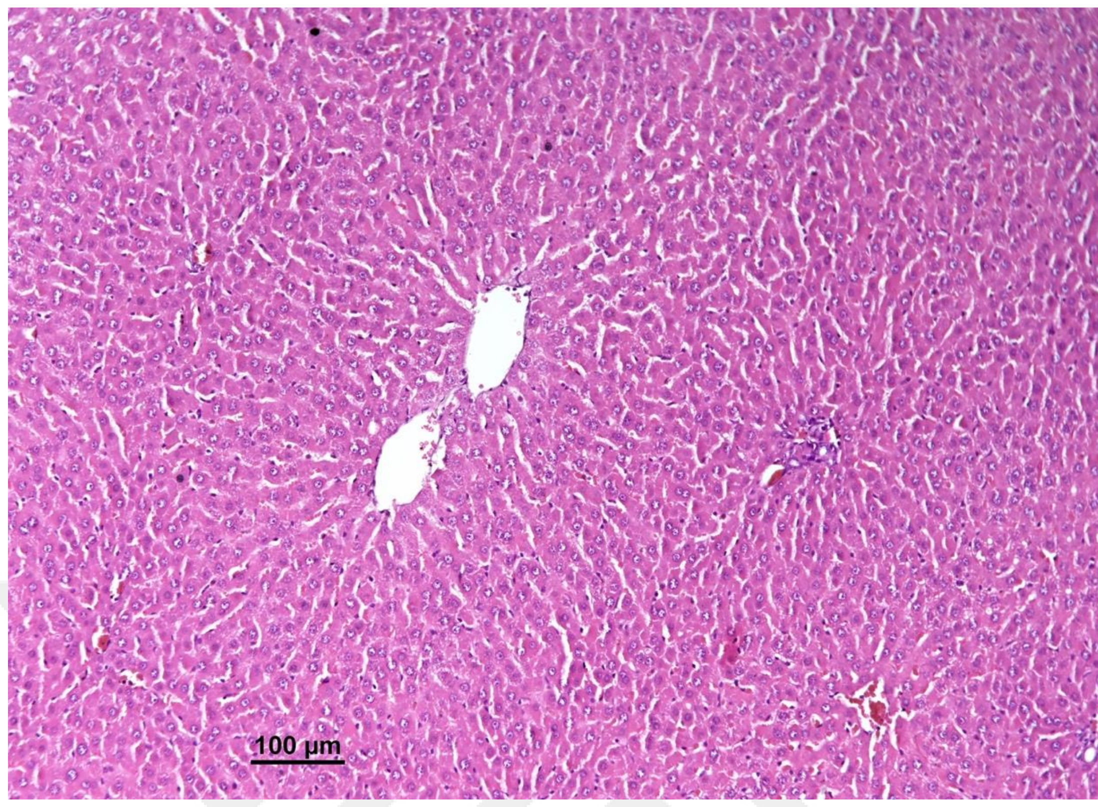

Figure 10. Control group: Normal appearance of the liver parenchyma.

In (mild, moderate and severe) groups exposed to liver injury with CCl_4 treatment, similar morphological changes were observed with different severity. These findings are scored in table 21. Hydropic degeneration and coagulation necrosis were seen in hepatocytes especially around vena centralis and mid-zonal regions. There was fibrosis in some vena centralis (Periacinary fibrosis) and portal areas. Radiation of fine collagen bundles from portal tracts into the parenchyma was only seen. Activation was also observed in perisinusoidal myofibroblastic cells (Figure 11, 12). Occasional accumulation of lymphoid cells intermixed with few macrophages were observed in the portal tracts and liver parenchyma. These changes resulted in dissociation of cells and degradation of the liver lobule architecture in the liver parenchyma of severe CCl_4 treatment group rats.

Table 21. Effects of CCl_4 on the liver tissue of rats

Changes/lesions in livers	Groups			
	Control	**Mild**	**Moderte**	**Severe**
Fibrosis	8/8 -	8/6 + 8/2 +	8/2 +++ 8/4 ++ 8/2 +++	8/5 +++ 8/2 +++ 8/1 ++
Degeneration	8/8 -	8/6 ++ 8/2 ++	8/2 ++++ 8/4 ++ 8/2 +	8/5 ++++ 8/2 ++ 8/1 ++
Necrosis	8/8 -	8/6 ++ 8/2 ++	8/2 +++ 8/4 ++ 8/2 +	8/5 +++ 8/2 ++++ 8/1 ++

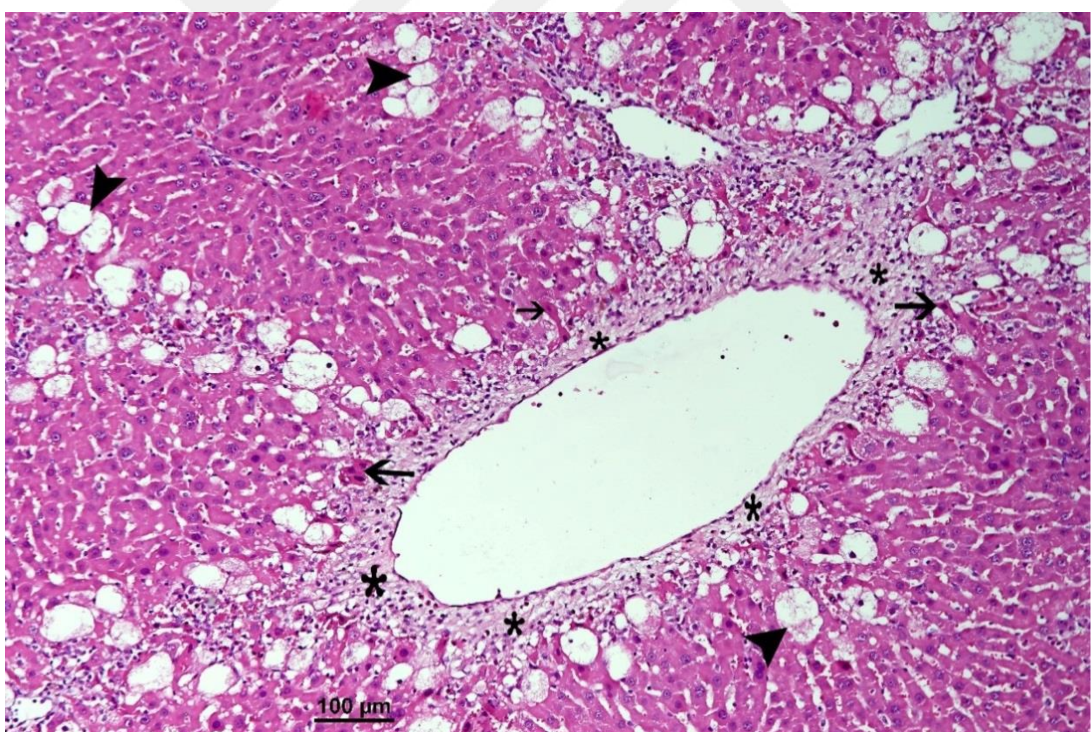

Figure 11. CCl_4 treatment group: Hepatocellular hydropic degeneration (arrow aheads) and necrosis (arrows) with the connective tissue increase in the centrilobular zone (∗).

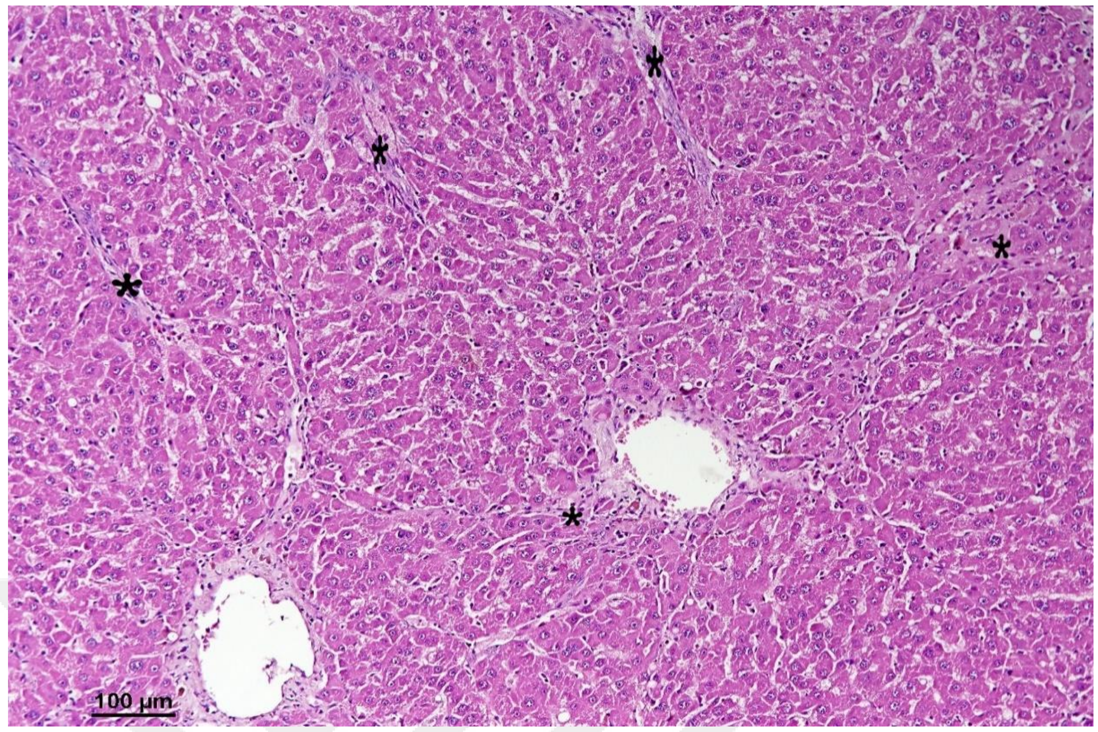

Figure 12. CCl_4 treatment group: The activation perisinusoidal and periacinary myofibroblastic cells (fine collagen bundles; fibrosis) (∗).

5. DISCUSSION

Liver is the largest and most complex internal organ of the body with multiple and various functions which plays an important role in protecting the internal section of body. This organ is the main organ for detoxification of many exogenous and endogenous toxic chemicals, and also it is main place for metabolism of materials like chemicals, drugs and other endogenous in the body (Vorobioff et al., 1983; Iredale, 2007; Fu et al., 2008). Hence in both human and veterinary medicine, the outbreak of major hepatic disorders like chronic and acute hepatitis, fibrosis, cirrhosis, liver cancers, fatty liver, has been taken to consideration and causes to lethal diseases (Luper, 1999; Wang et al., 2008; Abdalla et al., 2013). Today, liver diseases is on of the most common diseases globally. Hence, for assess the success and achieves to early diagnosis as well as prognosis of the treatment, alternative diagnostic methods and additional parameters are necessary. According to this, in addition to the methods and parameters used in the diagnosis and prognosis of hepatic problems, this study was objective to disclose the kallistatin serum levels alteretions in rats with different degrees of liver injury.

Carbon tetrachloride (CCl_4) is one of the most commonly hepatotoxin drugs which is used in experimental hepatopathy studies (Naik et al., 2007). Carbon tetrachloride is a potent hepatotoxic agent that causes hepatic necrosis, hepatic fibrosis as a result of centrilobular necrosis and is widely used in animal models for the induction of acute and chronic liver injury (Weber et al., 2003; Altaş et al., 2011; Ilavenil et al., 2012). This drug has been widely used in rodents as a model of studying and monitoring the mechanisms of hepatic injury (Wang et al., 2003; Yoshiji et al., 2003; Fang et al., 2004). In the present study, when hematological, biochemical and histopathological findings of rats-induced by different doses of CCl_4 and different liver damage were evaluated, it was found that CCl_4 is a good hepatotoxic agent for liver injury as reported by many researchers (Wang et. al., 2003; Weber et al., 2003; Yoshiji et al., 2003; Fang et al., 2004; Naik et al., 2007; Altaş et al., 2011; Ilavenil et al., 2012).

Liver-specific enzymes and hematologic parameters have been applied as indicators for the designation of hepatotoxic and hematotoxic effects of pesticides (Celik and Suzek, 2008). It has been reported that chlorpyrifos (as an organophosphate pesticides), applied in rats can cause significantly increase WBC and platelet counts, and in this respect,

hepotoxic agents could induce hematological changes in experimental animals (Yehia et al., 2007; Uzun and Kalender, 2013).

Saba et al. (2010), reported that in rats induced with CCl_4, RBC and Hb concentrations were decreased and microcytic hypochromic anemia was observed. In another study stated that using CCl_4 induces leukocytosis (Saba et al., 2010; Berezi et al., 2013). In studies on rats where CCl_4 was used orally, RBC, Hb and PCV values were reported to be decreased, and all hematological parameters were greatly affected (Mandal et al., 1998; Abdalla et al., 2013). Mortiz and Pankow (1989), in the study of the effects of CCl_4 on hematological parameters were indicated that acute CCl_4 toxicity leads to a transient decrease in Hb concentration and RBC. In CCl_4-induced rats, Hb concentration and RBC count (31% , 25%, $p<0.01$), respectively caused a significant decrease compared to the control group. Amer et al. (2015), reported that in healthy rats RBC, WBC, Platelet counts were ($5.61 \pm 0.95\ 10^6$ / mm^3), ($5.56 \pm 0.98\ 10^3$ / mm3) and ($718.60 \pm 52.72\ 10^3$ / mm^3), respectively, while RBC, WBC, and Platelets counts ($2.17 \pm 0.82\ 10^6$ / mm^3), ($9.19 \pm 1.12\ 10^3$ / mm^3), and ($403.20 \pm 50.21\ 10^3$ / mm^3), respectively in carbon tetrachloride-induced liver injury group were reported. Increased WBC count and high plasma WBC in CCl_4-induced fibrotic rats may be a diagnosis of the presence of pathogenic antigens in the systemic circulation (Ojiako et al., 2018).

White blood cells, which are indicated systemic inflammation, are generally checked out in clinical examinations. Measured WBC is in a relationship with several diseases like infection, cardiovascular disease, metabolic syndrome and liver damages (Lee et al., 2010; Chung et al., 2016; Jie et al., 2018). Connection between liver related problems and serum WBC counts has been identified in some studies. Studies have been indicated that WBC counts are related with the risk of occurring hepatic diseases. Therefore, counting of serum WBC could be useful in a process of detecting liver diseases (Lee et al., 2010; Chung et al., 2016; Jie et al., 2018). Cellular and soluble components of blood can be affected by liver synthetic dysfunction which can have adverse effect like haemolysis of acanthocytes and anemia due to abnormal lipid composition of the red blood cell membrane (Gonzalez-Casas et al., 2009; Marks, 2013). Procoagulant and anticoagulant proteins are initially synthesized in the liver. At the beginning of liver disease, coagulopathy associated with a prothrombotic state may

can happen, but by the progress of disease, blood coagulation disorder associated with haemorrhage could be manifested, therefore the bleeding disorders related to hepatic disease is complex (Gonzalez-Casas et al., 2009; Marks, 2013; Fortea et al., 2018). Studies indicated that Platelet to white blood cells ratio could be a helpful biomarker to detection of severity of liver related disorders (Chen et al., 2016; Jie et al., 2018). That is to say, decrease the ratio of Platelet to White Blood Cells may be an indicator of progression of the liver disease to worse condition, which is due to body's comprehensive response to infection (Weng et al., 2005; Chen et al., 2016; Jie et al., 2018).

In this study, according to the statistical analysis of haematological parameters (Table 7.), WBC levels were significantly lower in the control group compared to other groups ($p<0.05$), but by comparison within the groups exposed to liver injury, WBC value was found to be the highest value in the group with moderate liver injury. Our data is come in line with previous studies which were mentioned. Lymphocyte (LYM) value in moderate group was higher and significant among other groups ($p<0.05$). And about monocyte (MON) there was a meaningful difference between severe group and other groups ($p<0.05$). And about RBC and mean platelet volume (MPV) values no significant difference were found (Table 7.). Hemoglobin (Hb) and mean corpuscular volume (MCV) values were both showed significant difference between severe group and other groups ($p<0.05$). However, there weren't any important difference between control group, mild group and moderate group ($p>0.05$). As the injury worsened, mean cell hemoglobin (MCH) value decreased and fount to be lowest in severe group and also there was meaningful deference between control group and groups exposed to liver injury ($p<0.05$). Also, there was no important difference between the mild group and moderate group in intragroup comparison ($p>0.05$). On the contrary, there was a meaningful difference between severe group and mild and moderate groups ($p<0.05$) (Table 7.). In the intragroup comparison plateletcrit (PCT) values was differed between control group and moderate and severe groups ($p<0.05$) but wasn't differ between control group and mild group, as well between moderate group and severe group ($p>0.05$) (Table 7.). By comparison within the groups exposed to liver injury and control group, mean corpuscular hemoglobin concentration (MCHC) value was decreased and the lowest was found in the group with severe liver injury. However, there was no

meaningful difference between control group and mild group as well between moderate group and severe group ($p>0.05$). While, there was a significant difference between the control group and moderate and severe groups as well between mild group and moderate and severe groups ($p<0.05$) (Table 7.). By comparison within the groups exposed to liver injury and control group, there was a significant difference between control group and mild and severe groups in hematocrit (Hct) value ($p<0.05$). However, there wasn't important different between control group and moderate group as well between mild group and severe group ($p>0.05$) (Table 7.). In the case of neutrophil (NEU) parameter, there was a significant difference between control group and groups exposed to liver injury ($p<0.05$), and the highest value was belonging to moderate group. There wasn't any importance between mild and moderate groups ($p>0.05$), while there was significant difference between severe group and mild and moderate groups ($p<0.05$) (Table 7.). In the present study, we found that MPV levels were not statistically significant despite the increase in mild, moderate and severe groups compared to the control group ($p>0.05$) (Table 7.). Our data is come in line with previous studies which was mentioned (Weng et al., 2005; Gonzalez-Casas et al., 2009; Lee et al., 2010; Marks, 2013; Chen et al., 2016; Chung et al., 2016; Fortea et al., 2018; Jie et al., 2018).

An increase in enzymatic activity has been claimed to be caused by liver damage induced by CCl_4. Hepatotoxicity role of CCl_4 in experimental animals has been studied extensively and is widely used as a model agent to induce liver damage by activation of free radicals. The toxicity mechanism of CCl_4 is probably due to conversion of CCl_4 to high toxic trichloromethyl free radical (CCl_3-) by P540 cytochromes in the hepatocytes, which in turn is responsible to hepatocytes damage and decrease in liver activity (Boll et al., 2001; Çetin et al., 2011). More over, a decrease in the synthesis of proteins and increased levels and activities of liver marker enzymes including AST, ALT and ALP which are normal indicators of hepatological changes have been reported (Uzun and Kalender, 2013; Rahmouni et al., 2017; Uroko et al., 2019). Also it has effect on detoxification and removal of toxic substances in the body indicated by an increase in total bilirubin concentrations and together with liver enzyms due to liver injury (Uroko et al., 2019). Serum enzymes like ALT, AST, LDH and ALP are frequently used parameters to examine liver dysfunction (Uzun and Kalender, 2013; Rahmouni et al., 2017). They reported that serum ALT, AST, ALP, GGT levels in rats induced by CCl_4

were significantly higher than control groups. They indicate that there is positive correlation between increased serum AST and ALT activities and the incidence and severity of histopathological hepatic lesions (Altaş et al., 2001). Moreover, the increase in serum LDH activity which is figured out in hepatic necrosis, possibly occur owing to enzyme leak into the blood flow (Mansour and Mossa, 2010). Other investigators have reported enzymatic activities of AST, ALT and LDH are susceptible to serological liver toxicity (Moriyama et al., 1990; Messerah et al. 2011; Uzun and Kalender, 2013; McGill, 2016; Rahmouni et al., 2017; Ničković et al., 2018; Uroko et al., 2019). According to many reserchers serum AST and ALT activities are indicate liver function. liver enzymes in CCl_4-induced rats were notably changed and serum AST and ALT activities elevated at $p<0.05$ compared to the control group; they state that these hepatic biomarkers are elevated in serum due to the release of enzymes from the damaged liver cells (Albokhadaim, 2016). liver activities can be checked by study of ALT, AST and ALP blood serum enzymatic changes which are found in a large amount in the cytoplasm. In a case of liver injury, these enzymes penetrate to the blood stream in harmony with the degree of hepatic injury and their elevated serum levels can be measured (Naik et al., 2007). likewise, serum activities of ALT, AST, GGT and LDH were notably elevated as well as the increase in total bilirubin in CCl_4 treated rats. It is reported that serum ALT, AST, GGT and LDH levels are sensitive indicators of liver activity and the elevate their serum levels is generally happened by hepatocellular malfunction and disruption of the integrity of hepatic cell membranes (Abdou et al., 2015). Serum AST, ALT, ALP and LDH levels, which are liver pathophysiological marker enzymes, were significantly increased in CCl_4-induced rats (Arici and Çetin, 2011; Ilavenil et al., 2012; Althnaian et al, 2013; El-Bahr, 2014; Al-Sultan and El-Bahr, 2015). Wang et al (1997) was claimed that CCl_4 causes elevated serum ALT and AST activities in liver cells due to cell membrane and mitochondrial damages. Similarly, in another study, serum levels of AST, ALT and GGT were notably elevated in severe liver damage induced by CCl_4 contrast to healthy group, and this was in a relation with progression of hepatocytes damage; It is stated that enzymes located in the hepatocyts cytoplasmic are released into the circulation in case of cellular damage (Brent and Rumack, 1993).

Blood serum biomarkers measment followed by their interpretation, is one of the standard methods of clinical examination of liver function. For example amongst other

biomarkers, increasing in ALT and AST blood levels which are indicating liver dysfunctions. According to Nord (1982), Cheng et al (2015) and Adewale et al (2014), liver biomarker levels in rats induced by CCl_4 increase and this finding is in line with other studies which are claimed that administration of CCl_4 can increase the level of liver enzymes (Obi et al., 1998; Wegwu et al., 2005; Patrick-Iwuanyanwu et al., 2007). One study has indicated that injection CCl_4 in rats can cause an increase in serum levels of liver enzymes such as AST, ALT and ALP (Prakash et al., 2008). Another study has claimed that the increase in liver enzymes resulting from the use of CCl_4 in rats is similar to the results of Prakash et al (2008) who detected notable increase of ALT and AST due to liver injury in rats induced by CCl_4 As a result of hepatocytes permeation and lack of liver functional integrity. More specifically, elevate in the serum amount of ALT which is representative of liver dysfunction (Lin and Huang, 2000; Adewale et al., 2014).

This enzymes are found in the hepatocytes cytoplasm and are depleted into the blood as a result of any injury in liver (Obi et al., 1998; Lin and Huang, 2000; Wegwu et al., 2005; Patrick-Iwuanyanwu et al., 2007). Today blood serum enzymes such as ALT, AST, and GGT activities are considered as indicators of liver injury (Mohamed et al., 2014; lee et al., 2019; Ragab et al., 2019). Any increase in serum ALT, AST, and GGT amounts in rats intoxicated by CCl_4, represent liver disease, as they discharge from liver into circulation, as a result of liver tissue injury (Rahman and Sultana, 2006; Mohamed et al., 2014; Ragab et al., 2019). Studies found that blood serum levels of GGT, LDH, ALT and AST in animals after CCl_4-induced, were remarkably increased when compared with the serum levels in the control group. Today's ALT and AST beside other biochemical enzymes which known as intracellular ones that can be found in the blood circulation after damage-mediated cellular membrane injury are widely applied as a reputable markers for detection liver cells damage induced by CCl_4 (Moriyama et al., 1990; Messerah et al. 2011; McGill, 2016; Ničković et al., 2018).

In this study we found that, ALT blood serum is in a meaningful difference between severe group and other groups (A, B), while there was no difference between mild, moderate and control groups (B) (Table 9.). We found that ALT blood serum concentration in liver injury groups (104.93±45.63 IU/L, 261.63±72.19 IU/L, and 1076.46±864.43 IU/L)

respectively were higher than control group. The most amount of ALT was belonging to the severe group ($p<0.001$) (Figure 3, Table 9.).

Also current study indicated that serum level of AST in CCl_4-induced groups (213.67±75.10 IU/L, 448.81±84.04 IU/L, and 968.86±218.11 IU/L) respectively increased as well as the most amount belonging to severe group ($p<0.001$) (Figure 4). There was a meaningful difference between severe group and other groups (A, B, C), and also between mild and moderate groups (B, C), while there was no difference between mild and control groups (C) (Table 10.).

We found that GGT serum level respectively in mild, moderate and severe group (2.00±0.75 IU/L, 2.12±0.99 IU/L, and 4.00±2.07 IU/L) were increased compare to control group (1.37±0.51 IU/L) and were highest in severe group ($p<0.05$), while there was no reliable difference between the mild and medium groups and the control group and also between themselves (Table 8, Figure 5).

Serum LDH was another biomarker which is not reputable to detect liver diseases however could be remarkably increased as a result of ischemic problems, hepatic disorders related with hemolysis, and some kind of tumors (Gitlin and Serio, 1992). We found that, LDH serum concentrations respectively increased in mild, moderate and severe group (804.50±82.12 IU/L, 1077.25±191.97 IU/L, and 2090.88±679.7I U/L). And the most amount of LDH was belonging to severe group ($p<0.001$) (Figure 6, Table 8.). There is a meaningful difference between severe group and other groups (A, B, C), and also between mild and control groups (B, C) as well between moderate and control groups (B, C). There was no difference between mild and moderate groups (B) (Table 12.). So far our data is in agreement with previous studies which was mentioned (Moriyama et al., 1990; Gitlin and Serio, 1992; McGill, 2016; Ničković et al., 2018).

In the current study, it was found that blood serum enzyme concentrations of ALT, AST, LDH and GGT of rats with mild, moderate and severe liver injury were be higher than the control group. In addition, there was a positive correlation between the increase in enzyme activity concentrations and liver damage and the highest increase in enzyme activities was detected in rats belonging to severe group. Findings of serum enzyme activities in current study are come in a line with other findings of the

researchers and support their data (Recknagel et al., 1989; Brent and Rumack, 1993; Wang et al., 1997; El-Bahr, 2014; Al-Sultan and El-Bahr, 2015).

Hepatic fibrosis is often happens as a result of kinds of hepatic damages like toxical material exposure (Duval et al., 2015). It is specified by the excess deposition of extracellular matrix unites and it cause liver malfunction, even cancer (Li et al., 2015; Dong et al., 2016). Hepatic fibrosis which could advance to liver cirrhosis, carcinoma, hypertension, causing enhancement of morbidity and mortality is consider as a vital health concern (Enomoto et al., 2014; Dong et al., 2016). Amongst all causes inducing hepatic injuries, toxical materials are one that shouldn't be neglected. The reasons hepatic injuries as a result of toxic materials are multi-reason such as toxical effects brought about by showing a response to metabolites, inflammatory effects, and misbalances among body defensive response and cell injury which will progress to fibrosis in the liver tissue (Dhouib et al., 2015; Xuan et al., 2016; Fortea et al., 2018). Liver fibrosis has been specified as a reaction to inflammation necrosis due to several reasons like internal and external harmful factors infection, poisoning, and gives rise to the stimulation of Kupffer cells, and hepatic stellate cells cause of decay of liver cords. Continuing necrosis of liver cells can overshadow the liver regeneration capacity which in turn could change to fibrotic connective tissue (Abraldes et al., 2006; Fortea et al., 2018). Reconstruction of the liver lobular parenchyma as well as development of connective tissue septum could be changed by the reason of progressing liver fibrosis. It is surrounded as a constructive knot of liver cells and anastomosis amid vascular canals, interconnecting central and portal vessels by fibrous tissue (Liu et al., 2012; Fortea et al., 2018). Cirrhosis is known as the final stage of diverse infectious, poisons and another types of liver damage (Runyon et al., 1991). Up until now, histopathological survey is known as a precise diagnostic assessment which is fulfilled by liver biopsy and it is the main diagnostic evaluation for hepatic cirrhosis (Liu et al., 2012; Fortea et al., 2018). However, in clinical examines, applying of liver biopsy has many restrictions. For example, blind liver biopsy which can result in false-negative outcomes and complications and sample errors (Chatamra and Proctor, 1981; Proctor and Chatamra, 1982; Fortea et al., 2018).

There are many indirect diagnostic tests for detection liver cirhosis, included either clinical findings (Desmyter et al., 2007; Regimbeau et al., 2008; Fortea et al., 2018), blood biochemical parameters, or endoscopy and ultra-sonographic findings (Masuda et al., 1994; Desmyter et al., 2007). It is proven that there is a correlative between inflammatory agents and hepatic fibrosis, this indicates that, there is a significant connection between stage and the level of fibrosis (Sogni et al., 1998; Fortea et al., 2018). Carbon tetrachloride as a toxic laboratory reagent, is universally used to generate hepatic damage and hepatic fibrosis and lead to cirrhosis. CCl_4 has been widely used in hepatic-related studies (Huang et al., 1998; Dong et al., 2016). As mentioned before CCl_4 is broadly employed for experimental hepatic injury induction in rat (Liu et al., 2012). Decreased activities of antioxidant enzymes, generation of free radicals and lipid peroxidation altogether as a result of using CCl_4, could bring about liver injury (Proctor and Chatamra, 1982; Runyon et al., 1991; Berent and Rumak, 1993).

Histological survey of liver tissues has shown that CCl_4 induces fibrosis, cirrhosis and hepatocarcinoma (Junnila et al., 2000). Studies show that CCl_4 brings about an alteration in lipid together with the raise of the inflammation complex, occurring fiber parts and collagen accumulation, and also increase the loss of liver cells (Dong et al., 2015; Fortea et al., 2018). Chronic hepatic injury induced by CCl_4 in rat's develops hepatic fibrosis and in terms of histological and biochemical be similar to hepatic cirrhosis in both human and animals (Úbeda et al., 2010; Althnaian et al, 2013). Hence, the rat model of hepatic cirrhosis has been helpful in investigating based on liver function for use in both human medicine and veterinary (Constandinou et al., 2005). Liver lesion and liver fibrosis caused by using CCl_4 as a toxic substance in laboratory animals. As liver lesion progresses and become chronic it change to Liver fibrosis and then consequence of that progress into hepatic cirrhosis and finally cause carcinoma (Dong et al., 2016). But, the toxicological mechanisms of CCl_4-induced hepatic injuries stay not totally clear. Some studies claim the cause of liver injury could be as a result of activates the production of toxic radicals because of effect of CCl_4 on the liver cells (Recknagel and Litteria, 1960). Using of CCl_4 can leads to infiltration of the inflammatory cells and the consequence of the hepatic malfunction finally progress to

fibrosis. Pathologic findings are directly depended to proliferation of connective tissue and fibrotic respond (Guo et al., 2013; Dong et al., 2016).

The effect CCl_4 has on the liver can be detected by the changing in blood serum enzyme amounts alongside the histopathology and clinical findings (Dong et al., 2016). There are many studies on the effect of CCl_4 on the making and progress of liver diseases (wolf et al., 1998; Türkdoğan et al., 2003; Kumar et al., 2009; Cheng et al., 2015). As a model of liver injury, induction of CCl_4 is widely applied and admitted model. It has been reported increased lipid peroxidation in liver fibrosis induced by CCl_4 be connected with reduction in antioxidant enzymes amounts and injury to the liver cells structures (like membrane and tiny cellular structures) (Tribble et al., 1987; Özdemirler et al., 2008; Arıcı and Çetin, 2011). The oxidative stress, brought about an enhancement in reactive oxygen species (ROS) construction, is the principle etiology of different hepatic disorders (Lu et al., 2003; Mehmetçik et al., 2008; Ničković et al., 2018) and the main mechanism of the hepatic disorder is firmly linked to ROS, and CCl_4, as a model of liver diseases, is related to this mechanism (Lu et al., 2012; Ničković et al., 2018). The mechanism of liver injury is defined by a continual stimulus for liver cells degeneracy by a tissue fibrotic microenvironment and inflammation. Liver cirrhosis indicates the last pathological result for the most of chronic hepatic disorders. Recent studies suggest that any stage of fibrosis could be reversed (Arthur, 2002; Xu et al., 2006; Cheng et al., 2015). This makes it significant to find reputable biomarkers for on time detection of hepatic diseases and further assessment of response to remedial process. The capability of liver serum biomarkers to function, help to put more accurate diagnosis among stages of hepatic injury, would add a great privilege (Arthur, 2002; Xu et al., 2006; Cheng et al., 2015). There are many studies on the effect of CCl_4 on the making and progress of liver diseases (wolf et al., 1998; Türkdoğan et al., 2003; Kumar et al., 2009; Cheng et al., 2015). In this study, we found that CCl_4-applied led to liver damage and progressed it to fibrosis (Figure 10, 11, 12 and Table 21.), hence our finding comes in line with previous studies which was mentioned (wolf et al., 1998; Arthur, 2002; Türkdoğan et al., 2003; Xu et al., 2006; Kumar et al., 2009; Lu et al., 2012; Cheng et al., 2015; Dong et al., 2016; Ničković et al., 2018).

Hepatic diseases developments through several pathological steps that differ from mild liver injury without fibrosis to severe liver fibrosis and cirrhosis (Bravo et al., 2001; Bataller and Brenner, 2005; Fallatah, 2014; Leeming et al., 2014; Nallagangula et al., 2017). As mentioned before evaluation of the level of hepatic malfunction is essential for detecting, treatment, and pursue both throughout therapy and after ending of therapy (Bravo et al., 2001; Bataller and Brenner, 2005; Leeming et al., 2014; Fallatah, 2014). Detecting degree of hepatic fibrosis is a basic stage in assessment of intensity of the liver injury. Clinicians can achieve to accurate diagnosis of liver diseases stage with perform liver biopsy, which is histologically determines the stage of hepatic injury (Nallagangula et al., 2017). But as is clear this procedure accompanying with pain, stress and side effects (Fallatah, 2014; Nallagangula et al., 2017). Therefore, there are other ways which can help to diagnoses. One of these methods, is the evaluation of biomarkers, which are divided into the direct markers of extracellular matrix turnover and indirect markers as a reflexion of hepatic diseases. To approach accurate diagnosis, it is important to perform all markers and it make no sense to rely on one marker (Knodell et al., 1981; Scheuer, 1991; Sebastiani and Alberti, 2006; Standish et al., 2006). However, measuring a marker alone may not be beneficial for effective management, in other words a mathematical equation that combines tests can be useful (Knodell et al., 1981; Scheuer, 1991; Sebastiani and Alberti, 2006; Standish et al., 2006). The accurate diagnosis of severe liver diseases depends on the histological inspection of liver tissue. There are multiple non-invasive ways for evaluating the level of liver fibrosis, which are frequently used in clinical practice (Arthur, 2002; Dienstag et al., 2003; Cheng et al., 2015). Some are comparable to liver biopsy and have been approved in different studies, and compared with hepatic biopsy, some of them consider to be very precise in the evaluation of hepatic fibrosis (Arthur,2002; Dienstag et al., 2003; Hui et al., 2007; Freeman et al., 2008). They are usually considered as the reference method for assessment correctness of non-invasive procedures. Increase in amount of extracellular matrix can happen as a result of development of liver dysfunction, and additionally to the emergence of new blood serum biomarkers at different stages of fibrosis, the serum levels of various biomarkers also vary (Chao et al., 1996; Wolf et al., 1999; Poynard et al., 2003; Cheng et al., 2015). Technique of using

biopsy in liver diseases has many limitations, such as sample mistakes. Sometimes blind liver biopsy can be reached to more than 20 % of false negatives (Nord, 1982).

Liver fibrosis is a expand changing which includes initially of raised deposition of collagen in the liver (Rojkind and Perez-Tamayo, 1983; Ala-Kokko et al., 1987). It has been known that Collagen forms approximately 1 mg/g of fresh rat liver tissue (80% belonging to I and III collagens, and the rest 20% belonging to types IV and V altogether) (Ala-Kokko et al., 1987). So forth, accurate measurement of collagen types is difficult, and therefore values measured by different labs show some discrepancies (Rojkind and Perez-Tamayo, 1983; Ala-Kokko et al., 1987). Some article have been claimed that the total liver collagen enhanced up to 6 to 8 times in liver cirrhosis including all collagen types (Rojkind et al., 1979; Rojkind and Perez-Tamayo, 1983; Ala-Kokko et al., 1987). Until now the principle reason of increased liver collagen cumulation in hepatic fibrosis are remain unclear. Many studies have shown that in hepatic fibrosis, the liver produces a lot of collagen, which can be due to the balance between destruction and production (Rojkind and Perez-Tamayo, 1983; Ala-Kokko et al., 1987). In this study, we found that the amount of collagen I was increased in mild, moderate and severe groups (9.36 ± 0.35 ng/L, 10.75 ± 0.68 ng/L and 13.33 ± 0.78 ng/L), respectively compared to the control group ($p < 0.001$) (Table 8.). Collagen I in our study it was found that there were significant differences between all groups, besides the highest serum level been reported belonging to severe group. There was a meaningful difference between control group and groups belonging to liver injury (D, B, C, A), and also between groups belonging to liver injury (A, B, C) (Table 13.). Also we showed that the concentration serum level of collagen III also was increased in groups with liver injury mild, moderate and severe groups (respectively 339.31±18.54 ng/L, 380.89±17.99 ng/L, and 488.84±30.44 ng/L) compared to control group ($p<0.001$). As well as the highest amount was found that belonging to severe group (Table 8.). There was a meaningful difference between control group and groups belonging to liver injury (D, B, C, A), and also between groups belonging to liver injury (A, B, C) (Table 14.). Our data is come in line with previous studies which was mentioned. In the current study, alterations in the serum levels of collagen come in line with the changes reported by the investigators (Rojkind and Perez-Tamayo, 1983; Ala-Kokko et al., 1987).

There are many diagnostic researches on hepatic function in case of liver diseases and some of them belonging to biochemical investigation more especially about serum kallistatin levels (Wolf et al., 1998; Huang et al., 2014; Cheng et al., 2015). According to some articles based on research in human and rats, a decrease in kallistatin serum level in liver fibrosis and liver cirrhosis has been evidenced (Wolf et al., 1998; Huang et al., 2014; Cheng et al., 2015). In this study serum kallistatin level in control group was (7.96±1.76 ng/ml). We detected a decreasing in kallistatin serum level respectively in mild, moderate and severe groups (6.48±0.18 ng/ml, 5.91±.024 ng/ml, and 5.12±0.01ng/ml), compare to control group ($p<0.001$). Also we showed that the lowest amount was belonging to severe group. According to table there is a significant difference between control group and groups exposed to liver disease, and also there is a significant difference between mild group and severe group. However there is not difference between mild group and moderate group, and also between moderate group and severe group (Table 8.). This data is in agreement with previous studies which was mentioned (Wolf et al., 1998; Huang et al., 2014; Cheng et al., 2015). In this study we found there is negative relation between kallistatin and other biochemical parameters, which means that with decrease in kallistatin level, other biochemical parameters were increased (Table 20.).

Histological changes in liver injury caused by CCl_4 are known as apoptosis, necrosis, steatosis, and mononuclear cell infiltration in the lobular area of damaged liver (Rao et al., 2001; Canbay et al., 2004; Friedman, 2005). Mechanism of CCl_4 toxicity in rodents after oral administration have been researched by many researchers. And studies based on rats as experimental models, have been proved that both using CCl_4 or diethylnitrosamine develops both liver cirrhosis and hepatocellular carcinoma (Zalatnai et al., 1994; Ferencz et al., 2005). It has been reported that after 17 weeks of oral gavage of CCl_4 in mice model, although hepatocellular carcinoma and fibrotic tissue develops clearly however, cirrhosis wasn't developed (Fujii et al., 2010). Irrespective of etiology, hepatic fibrosis is a common pathological process of chronic liver injury and its progression leads to cirrhosis and liver cancer (Peng et al., 2009). Hepatotoxins like CCl_4, leads to liver necrosis, fibrosis, and cirrhosis when frequently used (Armendariz-Borunda et al., 1990; Kamalakkannan et al., 2005). The end stage of liver fibrosis is specified by cirrhosis, the formation of septa and nodes and altered bloodstream. Due to

synthesis and excessive accumulation of extracellular matrix (ECM) in the disse area (or perisinusoidal space), it leads to destruction of the structure along with insufficient ECM degradation an consequensly a gradual decrease in hepatic function. The process of repairing tissue damage is the normal response of the tissue to an injury, and liver fibrosis occurs as a result of repeated injury and repair cycles. Beside, chronic persistent inflammation typically precedes fibrosis. Chronic liver injuries activate stagnant hepatic stellate cells (HSCs) and convert them into activated myofibroblasts, the central pathogenic mechanism of fibrotic disorders (Friedman, 2003; Ebrahimkhani et al., 2008). In this study, in the histopathology examination of liver tissue; there was no histological change in the livers of the control rat. Our results showed that bile duct, parenchymal cells and Kuppfer cells were normal. Hepatocyte necrosis, inflammatory cell infiltration and bleeding foci were detected in CCl_4 -induced groups. Histological findings, changes in serum biochemical and hematological parameters of rats with moderate and severe liver damage induced by CCl_4 are evidence of serious cell damage in liver tissue. This data supports the data of other researchers (Armendariz-Borunda et al., 1990; Zalatnai et al., 1994; Friedman, 2003; Kamalakkannan et al., 2005; Ferencz et al. al., 2005; Ebrahimkhani et al., 2008; Peng et al., 2009; Fujii et al., 2010).

As it is known, hepatocytes have extensive activities, and due to the significance of liver fibrosis and cirrhosis; it is important to evaluate residual hepatic functional reserve, but a single marker is not fully trustworthy to estimate the rest of the their function (Huang et al., 2014). Recent research has shown that kallistatin, as a type of plasma protein belonging to the serine protease inhibitor family, is thoroughly connected with cellular adaptation to anti-inflammatory response and oxidative stress. Kallistatin serum amounts are lower in the kidney and blood vessels under conditions of oxidative stress; in one study in rats depletion of endogenous kallistatin by using anti-kallistatin antibody caused renal and cardiovascular oxidative stress and increased inflammation (Liu et al., 2012). As previously mentioned, the major site for production and secretion of kallistatin is the liver cells. It protects the liver organ against cell inflammation, fibrosis and oxidative stress (Cheng et al., 2015). According to researches in human medicine; serum kallistatin amounts are decreased in patients with hepatocellular carcinoma and liver cirrhosis and there is an inverse relationship between oxidative stress and kallistatin levels (Thongboonkerd et al., 2002; Cheng et al., 2015). Kallistatin is a

pleiotropic cytokine that has anti-inflammatory and anti-oxidant properties and can promise therapeutic in the prevention of various diseases including cardiometabolic disorders, vascular injury, arthritis, cancer, renal fibrosis, heart hypertrophy and fibrosis (Miao et al., 2002; Wang et al., 2005; Gao et al., 2008; Shen et al., 2008; Hsieh et al., 2009; Yin et al., 2010; Liu et al., 2012; Zhu et al., 2013; Huang et al., 2014). A study by Diao et al. (2011) on using kallistatin-encoding plasmid gene in mice, it showed that CCl_4 administration cause oxidative stress and inflammatory response were notably decreased, bringing about a reduction in the development of liver damage in mouse models. In the study performed in human medicine, it is stated that there is a significant decrease in kallistatin levels (7.2 ± 2.5 mg/mL, $p<0.001$) in plasma samples of patients with liver disease (Chao et al., 1996). To the best of our knowledge, This is the first study to show that the measurement of serum kallistatin concentration is a potentially useful marker in rats with liver injury induced with different doses of CCl_4. This study demonstrates that serum kalistatin amounts are markedly lower in rats belonging to study groups (different degrees of liver injury induced by CCl_4) compared to healthy group. And also, it indicates that as the intensity of hepatic injury develops, the serum kallistatin levels decrease. Therefore, according to data, it is concluded that serum kallistatin levels may be useful in response to therapy in liver related disorders or applying as an additional biological marker for detecting progressive liver failure.

The significant of the current study is to evaluate change in the level of kallistatin and other biochemical parameters in liver disease in rats. Despite liver, Kallistatin level change was implicated to damages in other organs including kidney and lungs, and protective role of this substance described by many studies (Miao et al., 2002; Wang et al., 2005; Gao et al., 2008; Shen et al., 2008; Hsieh et al., 2009; Yin et al., 2010; Diao et al., 2011; Liu et al., 2012; Zhu et al., 2013; Huang et al., 2014). It was approved that the administration of kallistatin has been attenuated the progression of liver disease (Diao et al., 2011; Huang et al., 2014; Cheng et al., 2015), so this study has tried to measure the change of the level of this substance beside evaluation of other liver enzymes in liver related diseases, which may be helpful for detecting kallistatin as biochemical marker for liver disease. And this may be a promising as a candidate drug for the prevention of liver disease, treatment of liver disease and may also as a treatment of other

inflammatory organs which are discussed previously, thus this study may add clues for kallistatin therapy/prevention approaches.

In this study we found that decreased serum kallistatin levels are associated with liver dysfunction. So that this finding can lead us to introduce kallistatin as a potential biomarker which can come to help for more accurate detection beside other diagnostic methods of liver disorders. And maybe it can help to early diagnosis but its needed further invastigation on this topic.

SUMMARY

Sepehrizadeh E., Evaluation of Kallistatin and some biochemical parameters in rat with experimental liver injury. Van Yuzuncu Yil University, Institute of Health Sciences, Department of Veterinary Internal Medicine, Ph.D.Thesis, Van, 2020. Kallistatin acts as a negative acute phase protein that reduces expression after lipopolysaccharide-induced inflammation in the liver. In this study, it was aimed to determine whether there is a relationship between the levels of Kallistatin and liver damage in rats with different degrees of liver failure. The material of this study consisted of 32 Wister Albino male rats obtained from Van Yüzüncü Yıl University Experimental Animals Production and Research Center and weighing between 200-400 gr were used. Thirty-two rats were included in 8 rats in each group as to group 1 (control, n=8), group 2 (mild, n=8), group 3 (moderate, n = 8), group 4 (severe, n=8). In order to cause experimental liver damage in rats, carbon tetrachloride (CCl_4) solutions of different percentages dissolved in paraffin oil were applied subcutaneously. Group 1: Healthy rats will not be treated with anything other than the normal diet. Rats in this group were named as control (n=8 male rats). Group 2: In this group (n=8 male rats), 25% CCI4 (dissolved in paraffin liquid) solution was applied to each rat at a dose of 2 ml / kg twice a week subcutaneously for 4 weeks. The rats in this group were named as mild group because of mild liver impairment. Group 3: 25% CCI4 (dissolved in paraffin liquid) solution was administered to rats in this group for 4 weeks in 2 ml / kg subcutaneously twice a week (as same as group 2) and the remaining 2 weeks were treated with 2 ml/kg of 50% CCl_4 (dissolved in paraffin liquid) solution (for a total of 6 weeks) and the rats in the group were named as the middle group (n=8 male rats) (Table 6). Group 4: the same procedure of group 3 had been applied for this group then proceed with injected subcutaneously of 2 ml/kg of 62.5% CCl_4 in paraffin oil treatment for the 2 weeks (on the whole 8 weeks) and this group was named as the severe group (n=8 male rats). Xylazine 60 mg / kg and Ketamine 7.5 mg / kg combinations were administered intramuscularly to rats in group 2 at the 4th week of the study, group 3 at in the sixth week of study, group 4 in the eighth week of the study and control group rats and 20-30 minutes from these applications. Then the rats were sacrificed. Blood samples were taken into anticogulant and non-anticogulant tubes for immediate hematological and biochemical analyzes in the heart of sacrified rats. Hematological parameters, hemoglobin concentration, leukocyte count, platelet count and MCHC values were measured on the same day with the Veterinary Hemogram Device (Veterinary MS4-s-Melet Schloesing Laboratories in France). Blood samples were taken into antiquagulant-free tubes for centrifugation at 3000 rpm for 15 minutes. The sera obtained were stored at -20 ° C until measurements were made. Serum AST, ALT, LDH and GGT levels were measured by autoanalyser (BS-120 Vet-Mindray) using commercial kits. Serum Rat Kallistatin (Rat Kallistan, Catalog NO: YLA1624RA SERINA4, ELISA kit Ylbiont), Rat collagen type I (Col I, Catalog NO: YLA0195RA, ELISA kit Ylbiont) and Rat collagen type III (Col III , Catalog NO: YLA0605RA ELISA kit Ylbiont) levels were measured using commercial ELISA test kits and with the ELISA instrument as specified in the procedure of the test kits. In the statistical analysis of hematological parameters, WBC, Neu, LYM, Mon, PCT levels in liver damage groups were significantly higher than the control group ($p<0.05$), while MCH and MCHC levels were lower ($p<0.05$). Although, RBC, Hct and MPV values were found to be mathematically high, and MCV and Hb values were mathematically low, they were not statistically significant ($p<0.05$). In the statistical analysis of biochemical parameters, in the analysis of serum ALT levels; serum ALT levels of rats with severe liver injury were found to be significantly higher than those of mild and moderate liver injury, whereas ALT levels of mild and moderate liver injury were compared with ALT levels of control group and no significant difference was found. In the analysis of serum AST levels; serum AST levels of rats with severe liver injury were significantly higher than those of rats with mild and moderate liver injury, but there was no significant difference between AST levels of mild liver injury and AST levels of control group. In the analysis of serum GGT levels; Serum GGT levels of rats with severe liver injury were found to be significantly higher than the GGT levels of mild and moderate liver injury rats, whereas there was no significant difference between liver injury groups (mild and moderate) and control group. In the analysis of serum LDH levels; serum LDH levels of rats with severe liver injury; was significantly higher than the LDH levels of rats with mild to moderate liver injury and control group, and also there were no significant difference between mild and moderate in rats belonging to liver injury groups. In the analysis of serum Collagen-I levels; Serum Collagen-I levels of rats with liver injury were significantly higher than those of the control group. Similarly, serum Collagen-III levels of rats with liver injury were significantly higher than those of the control group. The comparison between the groups was statistically exposed a significant difference between the kallistatin levels of mild liver injury and the kallistatin levels of severe liver injury, however there was no difference was found between the kal levels

of moderate and severe liver injury groups. Histopathological examination of the liver organ of the liver and control group rats revealed that the liver tissue had normal histological appearance, while the liver tissue of carbontetra chloride (in rats belonging to liver injury groups) showed severe morphological changes. These findings were hydropic degeneration and coagulation necrosis was detected in hepatocytes, especially in the vena centralis and mid-zone regions. Fibrosis was present in some vena centralis (Periacinary fibrosis) and portal areas. The spread of thin collagen bundles from the portal marks to the parenchyma was observed only. Activation was also observed in pericinusoidal myofibroblastic cells. Lymphoid cells mixed with several macrophages were observed to accumulate occasionally in portal scars and liver parenchyma. These changes resulted in cell dissociation and disruption of the liver lobe architecture in the liver parenchyma of heavy CCl_4 treatment group rats. When the histopathological examination of the hematological, biochemical parameters and liver tissue of rats with different degrees of liver damage were made with carbontetra chloride, significant changes were found in the control group. The relationship between the severity of liver tissue damage and the Kallistatin levels of the liver tissue of rats with carbontetra chloride (liver injury groups) was demonstrated by both biochemical and hematological parameters. As a result; Changes in the Kallistatin levels of rats with liver injury were found to be significantly low in parallel with the damage to the liver tissue. It was concluded that changes in serum kallistatin levels are an important parameter in determining liver tissue damage levels and evaluating treatment prognosis in the treatment of liver diseases.

Key Words: Kallistatin, Liver injury, CCl_4, Biochemical parameters

ÖZET

Sepehrizadeh E., Deneysel karaciğer hasarı oluşturulan ratlarda kallistatin ve bazı biyokimyasal parametrelerin düzeyleri. Van Yüzüncü Yıl Üniversitesi Sağlık Bilimleri Enstitüsü, Veteriner İç Hastalıkları Anabilim Dalı, Doktora Tezi, Van, 2020. Kallistatin karaciğerde lipopolisakkarid kaynaklı inflamasyon sonrası ekspresyonu azaltan negatif akut faz protein olarak görev yapmaktadır. Bu çalışmada, farklı derecelerde karaciğer yetmezliği oluşturulan ratlarda kallistatin düzeyleri ile karaciğerdeki hasarın dereceleri arasında ilişkinin olup olmadığının ortaya konulması amaçlandı. Hayvan materyali olarak; Van Yüzüncü Yıl Üniversitesi Deney Hayvanları Üretim ve Araştırma Merkezi'nden temin edilen ve canlı ağırlıkları 200-400 gr arasında değişen 32 adet Wister Albino erkek rat kullanıldı. Çalışmaya alınan 32 rat her grupta 8 rat olmak üzere kontrol (grup1, n=8), grup 2 (hafif, n=8), grup 3 (orta, n=8) ve grup 4 (şiddetli, n=8) olarak sınıflandırıldı. Ratlarda deneysel karaciğer hasarı oluşturmak için parafin yağında çözündürülen değişik yüzdelik oranlarında karbon tetraklorür (CCl4) solüsyonları subkutan uygulandı. Grup 1: Sağlıklı ratlara normal beslenme programı dışında herhangi bir şey uygulanmadı ve bu gruptaki ratlar kontrol (n=8 erkek sıçan) grubu olarak adlandırıldı. Grup 2: Bu gruptaki ratlara (n=8 erkek sıçan), % 25 CCI4 (parafin likitte çözdürülmüş) solüsyonu her ratta 2 ml/kg dozda, subkutan olarak haftada iki kez ve 4 hafta boyunca uygulandı. Bu gruptaki ratlar hafif derecede karaciğer bozukluğunu şekillendiği için hafif grup olarak adlandırıldı. Grup 3: Bu gruptaki ratlara ilk dört hafta % 25 CCI4 (parafin likitte çözdürülmüş) solüsyonu her ratta 2 ml/kg dozda subkutan olarak haftada iki kez ve 4 hafta boyunca uygulandı ve kalan 2 hafta % 50 CCl4 (parafin likitte çözdürülmüş) solüsyonu 2 ml / kg subkutan olarak 2 hafta boyunca uygulandı (toplamda 6 hafta boyunca) ve gruptaki ratlar orta grup (n=8 erkek sıçan) olarak adlandırıldı. Grup 4: bu grup için aynı grup 3 prosedürü uygulanmış ve daha sonra 2 hafta boyunca parafin yağı tedavisinde 2 ml / kg % 62.5 CCl4 subkütan yoldan enjekte edildi (toplamda 8 hafta boyunca) ve bu gruptaki ratlar şiddetli grup (n=8 erkek sıçan) olarak adlandırıldı. Çalışmanın 4. haftasında grup 2'deki, 6. haftasında grup 3'deki ve 8. Haftasında grup 4'deki ve kontrol grubundaki ratlara ksilazin 60 mg/kg ve Ketamin 7.5 mg/kg kombinasyonları kas içi uygulandı ve bu uygulamalardan 20-30 dakika sonra ratlar sakrifiye edildi. Sakrifiye edilen ratların kalbinde hemen hematolojik ve biyokimyasal analizler için antikogulantlı ve antikogulantsız tüplere kan numuneleri alındı. Hematolojik parametrelerin analizi aynı günde antikoagulantlı tüplere alınan kan örneklerinden, hematokrit değer, hemoglobin konsantrasyonu, lökosit sayısı, trombosit sayısı ve MCHC değerleri İç hastalıkları Anabilim dalımız laboratuvarında bulunan Veteriner Hemogram cihazı (Veterinary MS4-s-Melet Schloesing Laboratories in France) ile ölçüldü. Biyokimyasal parametrelerin ölçümü için antikuagulantsız tüplere alınan kan örnekleri 3000 devir/dk 15 dakika santrifüje edilerek serumları çıkarıldı. Elde edilen serumlar ölçümler yapılıncaya kadar -20 °C'de muhafaza edildi. Serum AST, ALT, LDH ve GGT düzeylerinin ölçümü için elde edilen serumlarda ticari kitler kullanılarak otoanalizatör (BS-120 Vet-Mindray) cihazıyla ölçümleri yapıldı. Serum Rat Kallistatin (Katalog NO: YLA1624RA SERİNA4, ELISA kit Ylbiont), Rat kollajen tip I (Col I, Katalog NO:YLA0195RA, ELISA kiti Ylbiont) ve Rat kollajen tip III (Col III, Katalog NO: YLA0605RA ELISA kit Ylbiont) seviyeleri ticari ELISA test kitleri kullanılarak ve test kitlerinin prosedüründe belirtildiği şekilde ELISA cihazıyla ölçümleri yapıldı. Hematolojik parametrelerin istatistiksel analizinde, karaciğer hasar gruplarına ait sıçanların WBC, Neu, LYM, Mon, PCT düzeyleri kontrol grubuna göre anlamlı derecede yüksek ($p<0.05$) tespit edilirken, MCH ve MCHC düzeyleri ise daha düşük saptandı (p.<0.05). Ancak RBC, Hct ve MPV değerleri matematiksel olarak yüksek ve MCV ve Hb değerleri matematiksel olarak düşük olmasına rağmen istatistiksel olarak anlamlı bulunmadı ($p<0.05$). Biyokimyasal parametrelerin istatistiksel analizinde, serum ALT düzeylerinin analizinde; Şiddetli karaciğer hasarı olan sıçanların serum ALT düzeylerinin hafif ve orta derecede karaciğer hasarı olanlardan anlamlı derecede yüksek olduğu bulundu. Hafif ve orta şiddette karaciğer hasar gruplarının ALT düzeyleri kontrol grubunun ALT düzeyleri ile karşılaştırıldı ve anlamlı bir fark bulunmadı. Serum AST düzeylerinin analizinde; şiddetli derecede karaciğeri hasarı oluşturulan ratların serum AST düzeyleri kontrol, hafif derecede ve orta derecede karaciğeri hasarı oluşturulan ratların AST düzeylerine göre anlamlı düzeyde yüksek bulunurken hafif derecede karaciğeri hasarı oluşturulan ratların AST düzeyleri ile kontrol grubunu AST düzeyleri arasındaki fark anlamlı bulunmadı. Serum GGT düzeylerinin analizinde; Şiddetli karaciğer hasarı olan sıçanların serum GGT düzeylerinin, hafif ve orta derecede karaciğer hasarı sıçanlarının GGT düzeylerinden anlamlı derecede yüksek olduğu, karaciğer hasarı grupları (hafif ve orta) ile kontrol grubu arasında anlamlı bir fark olmadığı bulundu. Serum LDH düzeylerinin analizinde; Şiddetli karaciğer hasarı olan sıçanların serum LDH seviyeleri; hafif ila orta şiddette karaciğer hasarı ve kontrol grubu olan sıçanların LDH düzeylerinden anlamlı derecede yüksek tespit edildi. Ayrıca hafif ve orta şiddette karaciğer hasarı grupları arasında anlamlı fark

bulunmadı. Serum kollajen-I düzeylerinin analizinde; Karaciğeri hasarı oluşturulan ratların serum kollajen-I düzeyleri kontrol grubunun kollajen-I göre önemli düzeyde yüksek tespit edildi. Benzer şekilde Karaciğeri hasarı oluşturulan ratların serum kollajen-III düzeyleri kontrol grubunun kollajen-III göre önemli düzeyde yüksek tespit edildi. Kallistatin düzeylerinin analizinde; serum kallistatin düzeyleri karaciğer hasarı oluşturulan tüm gruplarda kontrol grubuna göre düşük bulundu. Gruplar arası karşılaştırmada ise şiddetli derecede karaciğeri hasarı oluşturulan ratların kallistatin düzeylerinin hafif derecede karaciğeri hasarı oluşturulan ratların kallistatin düzeylerine göre anlamlı düzeyde düşük tespit edildi. Orta derecede karaciğeri hasarı oluşturulan kallistatin düzeyleri hafif derecede karaciğeri hasarı oluşturulan ratların kallistatin düzeyleri ile arasında fark bulunmadı. Kontrol grubu ratların ile Karaciğer hasarı oluşturulan ratlarının karaciğer organın histopatolojik incelenmesinde kontrol grubunun ratların karaciğer dokusunun histolojik görünümü normal görünümde olduğu saptanırken, karbontetra klorürle karaciğer hasarı oluşturulan ratların karaciğer dokusunda şiddetli derecede morfolojik değişiklikler saptandı. Karaciğer organın özellikle vena centralis ve orta-zon bölgelerinde hepatositlerde hidropik dejenerasyon ve pıhtılaşma nekrozu tespit edildi. Bazı vena centralis (Periacinary fibrosis) ve portal alanlarda fibroz yapı mevcut, ince kollajen demetlerinin portal izlerinden parankime yayılması ve aktivasyon, perisinusoidal miyofibroblastik hücrelerde de gözlendi. Karaciğer portal izlerinde ve karaciğer parankimi içinde birkaç makrofaj ile karışmış lenfoid hücrelerin zaman zaman biriktiği gözlendi. Bu değişiklikler, hücrelerin ayrışması ve ağır CCl4 tedavi grubu sıçanlarının karaciğer parankiminde karaciğer lob mimarisinin bozulması ile sonuçlanmıştır. Karbontetra klorür ile değişik derecelerde karaciğer hasarı oluşturulan ratların hematolojik, biyokimyasal parametreler ve karaciğer dokusunun histopatolojik incelenmesi kontrol grubuna göre değerlendiriliğinde önemli düzeyde değişiklikler saptandı. Karbontetra klorürle karaciğer hasarı oluşturulan (karaciğer hasarı gruplarında) ratların karaciğer dokusundaki hasarın şiddeti derecede morfolojik değişiklikler ile kallistatin düzeyleri arasında bir ilişkinin olduğu hem biyokimyasal hemde hematolojik parametrelerle ortaya konuldu. Sonuç olarak; Karaciğeri hasarı oluşturulan ratların kallistatin düzeylerindeki değişimleri ile karaciğer dokusunda oluşan hasara paralel olarak önemli düzeyde düşük tespit edildi. Serum kallistatin düzeylerindeki değişimlerin karaciğer doku hasar düzeylerinin belirlenmesinde ve karaciğer hastalıklarının tedavisinde uygulanan tedavi prognozunun değerlendirilmesinde önemli bir parametre olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Kallistatin, Karaciğer hasarı, CCl_4, Biyokimyasal parametrelerin

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APPENDIX

EK 1. Etik Kurul Onay Belgesi

T.C.
VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU

ARAŞTIRMA BAŞVURU ONAY BELGESİ

	Araştırmanın Adı	Deneysel Karaciğer Yetmezliği Oluşturulan Ratlarda Kallistatin ve Bazı Biyokimyasal Parametrelerin Değerlendirilmesi
	Araştırmanın Yürütücüsü	Prof. Dr. Süleyman KOZAT
	Yardımcı Araştırıcılar	Ehsan SEPEHRİZADEH
	Kurumu	Veteriner Fakültesi
	Araştırmanın Tahmini Süresi	22 Ay
	Kullanılacak Hayvan Türü ve Sayısı	Sıçan 32 Adet
	Destekleyecek Kuruluş (lar)	VAN YYÜ BAP Başkanlığı
	Başvuru Tarihi	24.10.2018

KARAR BİLGİLERİ	**Karar No:2018/11** **Tarih:29.11.2018** Van Yüzüncü Yıl Üniversitesi Veteriner Fakültesi öğretim üyesi/elemanı Prof. Dr. Süleyman KOZAT sorumluluğunda yürütülmesi planlanan ve yukarıda başvuru bilgileri verilen doktora projesi, gerekçe, amaç ve yöntemler dikkate alınarak ilgi başvuru belgeleri incelendi. Çalışmanın etik açıdan uygun olduğuna, projenin aşağıdaki hususlar dikkate alınarak yürütülmesine ve proje yürütücüsüne iletilmesine oy birliği / oy çokluğu ile karar verildi. 1) Projede herhangi bir değişiklik gerektiğinde kurulumuzdan onay alınması. 2) Projede çalışacağı bildirilen araştırıcılarda değişiklik olduğunda kurulumuzdan onay alınması. 3) Deney hayvanları üzerinde yapılacak girişimin başlangıç ve bitiş tarihlerinin bildirilmesi. 4) Çalışma süresinde tamamlanamaz ise ek süre talebinde bulunulması. 5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.

	BAŞKAN/CHAIR Prof. Dr. Semiha DEDE	
ÜYE Prof. Dr. N. Tuğba BİNGÖL	**ÜYE** Prof. Dr. Sıddık KESKİN	**ÜYE** Prof. Dr. Suphi DENİZ
ÜYE Prof. Dr. Nalan ÖZDAL	**ÜYE** Doç. Dr. Atilla DURMUŞ	**ÜYE** Doç. Dr. Yıldıray BAŞBUĞAN
ÜYE Doç. Dr. Ferda KARAKUŞ	Dr. Öğr. Üyesi. Oruc ALLAHVERDİYEV	**ÜYE** Dr. Öğr. Üyesi Canser Yılmaz DEMİR
ÜYE Dr. Öğr. Üyesi Hacer ŞAHİN AYDINYURT	**ÜYE** Dr. Öğr. Üyesi Şükrü ÖNALAN	**ÜYE** Vet. Hek. Kerem OĞRAK
ÜYE Vet. Hek. İsmail Hakkı BEHÇET	**ÜYE** Zir. Müh. Kenan YILDIRIMOĞLU	

***Bu form VAN YÜHADYEK tarafından doldurulacaktır.**

EK 2. Etik kurul Araştırma Kesin Sonuç Onay Belgesi

VAN YÜHADYEK
VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

ARAŞTIRMA KESİN SONUÇ ONAY BELGESİ

VAN YUZUNCUYILUNIVERSITY (TURKEY)
ANIMAL RESEARCHES LOCAL ETHIC COMMITTEE
RESEARCH FINAL REPORT APPROVAL CERTIFICATE

Araştırmanın Adı *Research Title*	Deneysel Karaciğer Yetmezliği Oluşturulan Ratlarda Kallistatin ve Bazı Biyokimyasal Parametrelerin Değerlendirilmesi *Evaluation of Kallistatin and some biochemical parameters in rat with experimental liver injury*
Araştırıcı(lar) *Investigator(s)*	**Yürütücü** / *Chief investigator : Prof. Dr. Süleyman KOZAT* **Yardımcı Araştırıcı(lar)** / *Co-investigator(s): Vet. Hek. Ehsan Sepehrizadeh*
Araştırmanın Başlama Tarihi / *Research Starting Date: 14.12.2018*	
Araştırmanın Bitiş Tarihi / *Research Completion Date: 10.10.2019*	
Proje Süresi / *Total Time of Project:24 ay (month)*	
Proje No / *Project Number: TDK-2019-7863*	
Araştırmayı Destekleyen Kuruluş (varsa)/ *Funding institution(s) (if available):BAP*	
Destek Şekli ve Miktarı / *Type and amount of funding: 16005,60 (TL)*	

Karar:
Yukarıda bilgileri verilen araştırma projesinin kesin sonuç raporu Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 31/10/2019 tarih ve 2019/10 sayılı kararı ile kabul edilmiştir.
Decision:
Final report of the research project detailed above was approved by Van Yuzuncu Yil University Animal Researches Local Ethic Committee in the session held on 31/10/2019 (decision number 2019/10)

	BAŞKAN/CHAIR Prof. Dr. Semiha DEDE	
ÜYE/Member Prof. Dr. N. Tuğba BİNGÖL	**ÜYE/Member** Prof. Dr. Sıddık KESKİN	**ÜYE/Member** Prof. Dr. Nalan ÖZDAL
ÜYE Prof. Dr. Atilla DURMUŞ	**ÜYE/Member** Doç. Dr. Ferda KARAKUŞ	**ÜYE/Member** Doç. Dr. Yıldıray BAŞBUĞAN
ÜYE/Member Doç. Dr. Canser Yılmaz DEMİR	**ÜYE/Member** Doç. Dr. Hacer ŞAHİN AYDINYURT	**ÜYE/Member** Dr. Öğr. Üyesi Oruç YUNUSOĞLU
ÜYE/Member Dr. Öğr. Üyesi Şükrü ÖNALAN	**ÜYE/Member** Vet. Hek. İsmail Hakkı BEHÇET	**ÜYE/Member** Zir. Müh. Kenan YILDIRIMOĞLU

EK 3. Tez orijinallik raporu

EK 3. Tez orijinallik raporu

T.C.
VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ
Sağlık Bilimleri Enstitüsü

DOKTORA TEZİ ORİJİNALLİK RAPORU

Tarih: 16/12/2019

Tez Başlığı / Konusu: **Evaluation of Kallistatin and Some Biochemical Parameters nn Rat with Experimental Liver Injury**

Yukarıda başlığı/konusu belirlenen tez çalışmamın Kapak sayfası, Giriş, Ana bölümler ve Sonuç bölümlerinden oluşan toplam 67 sayfalık kısmına ilişkin, 04/12/2019.. tarihinde şahsım/tez danışmanım tarafından turnitin..intihal tespit programından aşağıda belirtilen filtreleme uygulanarak alınmış olan orijinallik raporuna göre, tezimin benzerlik oranı %17 (onyedi) dır.

Uygulanan filtreler aşağıda verilmiştir:

- Kabul ve onay sayfası hariç,
- Teşekkür hariç,
- İçindekiler hariç,
- Simge ve kısaltmalar hariç,
- Gereç ve yöntemler hariç,
- Kaynakça hariç,
- Alıntılar hariç,
-Tezden çıkan yayınlar hariç,
- 7 kelimeden daha az örtüşme içeren metin kısımları hariç (Limit match size to 7 words)

Van Yüzüncü Yıl Üniversitesi Lisansüstü Tez Orijinallik Raporu Alınması ve Kullanılmasına İlişkin Yönergeyi inceledim ve bu yönergede belirtilen azami benzerlik oranlarına göre tez çalışmamın herhangi bir intihal içermediğini; aksinin tespit edileceği muhtemel durumda doğabilecek her türlü hukuki sorumluluğu kabul ettiğimi ve yukarıda vermiş olduğum bilgilerin doğru olduğunu beyan ederim.

Gereğini bilgilerinize arz ederim.

Öğrencinin Adı Soyadı
İmza
Ehsan Sepehrizadeh

Öğrencinin Adı Soyadı		Ehsan sepehrizadeh	
Anabilim Dalı	:	Veterner Iç Hastalıkları	
Öğrenci No		149301034	
Programı	:	☐ Yüksek Lisans	☒ Doktora

DANIŞMAN ONAYI
UYGUNDUR
Prof. Dr. Süleyman KOZAT

ENSTİTÜ ONAYI
UYGUNDUR
(Doç. Dr. Hamit Hakan ALP)