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Evaluation of kallistatin and some biochemical parameters in rats with experimental liver injury

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ABSTRACT

This study aims to determine the possible relationship between the levels of Kallistatin and the severity of liver injury in rats that were exposed to carbon tetrachloride (CCl4). According to the results, serum ALT, AST, LDH, GGT and Collagen-I and Collagen-III levels in rats in the severe group were higher than control. Histopathological examination of rats in injury groups showed severe morphological changes that were resulted in cell dissociation and disruption of the liver lobe architecture in the liver parenchyma of rats that received CCl4. Kallistatin serum level decreasing respectively in mild group (M1), moderate group (M2), and severe group (S) groups compared to the control group, and the lowest amount was belonging to the severe group. As a result, there was a reverse connection between Kallistatin serum level and the liver injury intensity. Serum kallistatin levels are an essential parameter in determining liver tissue damage levels, and measuring it may help provide a treatment prognosis.

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Introduction

Liver is the largest organ in the body; this organ has more biochemical functions than other body organs, mainly consisted of hepatocytes, sinusoidal cells, and biliary epithelium (Vdoviaková, Petrovová, Maloveská, 2016; Kruepunga et al., 2019). The liver has specific functions, such as removing exogenous and endogenous toxins and synthesizing vital substances like blood clotting agents, albumin, and enzymes. It is also played a fundamental role in the metabolism of proteins, fats, carbohydrates, storage of vitamins and minerals, the process of conjugation and excretion of bilirubin in the bile, activation of glycogen and triglyceride, various hormones balance, and generating bile salts (Vdoviaková, Petrovová, Maloveská, 2016).

The complexity of liver dysfunction and the influence of many environmental factors on the appearance of the disease and response to treatment indicate the need to study serum-based function to find markers that can be sensitive, accurate, diagnostic, and prognostic (Cheng et al., 2015). Elevated liver enzymes, a conjugated or separated changing of biochemical markers in patients with liver abnormalities, are a massive reason for debate among clinicians. These serum biochemical enzymes are considered as one of the most common laboratory challenges which can make a mistake during the identification of liver diseases (primary belonging to hepatic problems or related to some other parts of the body, secondary due to extrahepatic issues). For this reason, it is important to find a new diagnostic method to help to solve this problem; therefore, this study aims to explore changes in the level of blood Kallistatin

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(as a possible specific diagnostic biomarker which is mostly produced in the liver) and some biochemical liver indicators like blood serum of ALT, AST, the 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 considered as a diagnostic indicator of healthy hepatic status or not (Cheng et al., 2015; Moreira et al., 2017). In some studies, it has been declared that the administration of kallistatin can decrease the progression of liver-related diseases (Cheng et al., 2015). Initially, based on other researchers, it was hypothesized that serum kallistatin levels could be a potential biomarker, and measuring its serum amount along with other liver parameters could be a useful approach for more accurate diagnosis of liver-related problems (Cheng et al., 2015; li et al., 2015).

Results from the current study, both in human medicine and veterinary medicine, could be useful in gathering more information about introducing new biomarkers for liver-related problems. Besides, it may also help studies based on introducing new biomarkers as a clue for the diagnosis and treatment of clinical liver diseases and other investigations focused on kallistatin effects.

Materials and Methods

Animal material: As the animal material of the study, 32 Wister Albino male rats with live weights ranging from 200-400 gr were used in the Experimental Animal Breeding and Research Center of Van Yuzuncu Yil University. The Animal Ethics committee approved animal experiments. The rats cared for according to protocols approved by the Animal Care and Use Committee. 7 days before the investigation; all rats were housed and acclimated to the environment at 75±2% relative humidity in a temperature-controlled environment (20-22 °C) and with a standard light / dark cycle.

The rats were randomly divided into four groups, and each group consisted of eight male rats. The groups were named below: Control group (group C), which was healthy, and it was given a regular standard pellet diet. The mild group (group M1) was injected subcutaneously with 2 ml/kg of 25% CCl_4 in paraffin oil (Sigma Co., Milan, Italy) twice a week for four weeks. Moderate group (group M2), the same procedure as the mild group was applied to this group (injected subcutaneously with 2 ml/kg of 25% CCl_4 in paraffin oil twice a week for four weeks), and to make a moderate group, the procedure was followed by subcutaneous injection of 2 ml/kg of 50% CCl_4 in paraffin oil for the two weeks (6 weeks in all). Severe group (group S), which CCl_4 was administered to this group in three stages with three different doses for a total of 8 weeks. Firstly, rats were subcutaneously applied twice a week for four weeks with a 25% CCl4 solution dissolved in paraffin oil at a dose of 2 ml/kg (as to mild group). Secondly, 50% CCl_4 solution dissolved in paraffin oil was applied subcutaneously twice a week for two weeks at a dose of 2 ml/kg (as to moderate group), and finally, 62.5% CCl_4 solution dissolved in paraffin oil was applied subcutaneously at a dose of 2 ml/kg twice a week for two weeks (Li et al., 2016).

At the end of the study, all rats were anesthetized with the administration of a combination of xylazine and ketamine (60 mg / kg+7.5 mg/kg), and then were sacrificed on the same day. Blood samples were directly gathered into tubes and then centrifuged at 3000 g for 15 min at 4◦C for serum preparation. Liver samples were prepared and washed with an ice-cold PBS to eliminate blood, and they were fixed in a 10% formalin solution for histopathological surveys.

Hematological analysis: Blood samples were taken from both posterior vena cava and direct heart puncture to analyze 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 tests. Measurement of hematological parameters: Lymphocyte (Lym) and monocyte (Mon), neutrophil (Neu), hematocrit value (Hct), hemoglobin concentration (Hb), white blood cell (WBC) count, red blood cell (RBC) count, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean platelet volume (MPV), plateletcrit (Pct), and mean corpuscular hemoglobin concentration (MCHC) were measured by veterinary hemogram device (Veterinary MS4-s-Melet Schloesing Laboratories in France).

Biochemical analysis: As mentioned, according to the method to be used in biochemical examinations, blood samples taken in anticoagulant-free tubes were centrifuged at 3000 rpm for 15 minutes. Then their serum was collected and stored at -20 °C for further analysis. ELISA (enzyme-linked immunosorbent assay) was done using a polyclonal antibody to rat kallistatin. ELIZA techniques were implemented for analyzing rat serum kallistatin levels and measured by commercial ELISA kits (YL biont, Shanghai YL biotech company, China) (catalog No: YLA1624RA) due to the producer's instructions. As well Rat COL-I (YL biont) (catalog No: YLA0195RA) and Rat COL-III (YL biont) (catalog No: YLA0605RA) was measured due to the producer's instructions (Li et al., 2015). In short, the kallistatin sample and standards were added, in duplicate, into a

96-well microtiter plate previously coated with none labeled anti kallistatin IgG. All plates had incubated at 37°C for 90 min; then, the 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 color reaction. The same technique was 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 auto-analyzer (BS-120 Vet-Mindray, Mindray Animal Care Co., Ltd., China)

Histopathological analysis: At the end of the experiment, the rats were necropsied following their euthanasia by anesthesia. Following the macroscopic examination, samples taken from the liver were detected in 10 % neutral formalin for histopathological examination. At the end of routine tissue follow-up, 4-5 microns thick sections taken from paraffin blocks were stained with hematoxylin-eosin and Masson trichrome. Semi-quantitatively; fatty, necrosis and fibrosis in hepatocytes in sections stained with hematoxylin-eosin were scored by two pathologists by taking the average value of each damage parameter by counting 10 and 20 lenses in 10 different areas in each slide.

Statistical analysis: Descriptive statistics were used for the properties of biochemical parameters of liver rats and healthy rats; Mean were expressed as Standard Deviation. 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.

Results

Hematologic findings: In statistical analysis, WBC and Neu values of rats with mild, moderate, and severe liver damage were significantly higher ($P < 0.05$) compared to the control group. The Lym and Mon values of the rats with moderate liver damage were significantly higher ($P < 0.05$) than the Lym and Mon values of rats with control, mild and severe liver damage groups. While Hct values of rats with mild liver damage were significantly higher ($P < 0.05$) than the Hct values of the control, moderate and severe liver damage groups, Hct and Hb values of the group with severe liver damage were significantly lower ($P < 0.01$) than the Hct and Hb values of the control, mild and moderate liver damage groups. MCV, MCH, and MCHC values of rats with severe liver damage were significantly lower ($P < 0.05$) compared to control rats with mild and moderate liver damage. Similarly, the MCH values of the rats with mild and moderate liver damage were significantly lower ($P < 0.05$) compared to the control group's MCH values. The Pct values of rats with moderate and severe liver damage were significantly higher ($P < 0.05$) compared to the Pct values of rats with mild and moderate liver damage (Table 1).

Table 1. Hematological parameters in control and liver damage of rat

Parameter	Control (Group1)	Mild liver disorder (Group 2)	Moderate liver disorder (Group 3)	Severe liver disorder (Group 4)
WBC (10^3/mm^3)	5.2 6 ± 1.8^a	13.4 ± 4.01^b	22.1 ± 11.07^b	7.2 0 ± 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	15.8 ± 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^a	0.47 ± 0.14^b	0.74 ± 0.23^b	0.28 ± 0.14^a
Hct (%)	39.0 ± 2.54^a	43.8 ± 3.81^b	40.9 ± 3.49^a	35.8 ± 9.35^b
Hb (g/dl)	16.7 ± 0.53^a	17.5 ± 1.75^a	15.2 ± 1.38^a	12.9 ± 3.70^b
MCV (fl)	57.6 ± 1.41^a	55.6 ± 1.18^a	55.5 ± 3.22^a	52.8 ± 6.09^c
MCH (fl)	24.7 ± 1.48^a	22.2 ±.94^b	20.7 ± 1.06^b	18.6 ± 2.30^c
MCHC (g/dl)	43.0 ± 2.19^a	40.03 ± 2.00^a	37.1 ± 1.66^c	35.4 ± 2.43^c
Pct (%)	0.31 ± 0.05^a	0.30 ± 0.14^a	0.52 ± 0.30^b	0.50 ± 0.20^b

Data are expressed as mean ± standard deviation. N number is 8 in each group. a, b, c: Different lower cases in the same column represent statistically significant differences ab, ac, bc: p<0.01 or p<0.05, and same lower cases in the same column represent statistically not substantial differences. aa, bb, and cc: P>0.05. WBC = White blood cell, RBC = Red blood cell, Neu = Neutrophil, Lym = Lymphocyte, Mon = Monocyte, HCT = Hematocrit, Hb = Hemoglobin, MCV = Mean cell volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, Pct = Plateletcrit

Biochemical findings: In the statistical analysis of biochemical parameters, serum ALT, AST, LDH, and GGT levels in rats with severe liver injury were significantly higher than those of the control group and rats with mild and moderate liver injury. ALT and GGT levels did not show any significant difference between rats belonging to control, mild, and moderate liver injury groups. LDH levels in rats with mild and moderate liver injury groups were higher than the control group.

Table 2. Biochemical parameters in control and rat with liver injury

Parameter	Control (Group1)	Mild liver disorder (Group 2)	Moderate liver disorder (Group 3)	Severe liver disorder (Group 4)
ALT (IU/L)	30.8 ± 4.2[a]	104.9 ± 45.6[a]	261.6 ± 72.1[a]	1076.46 ± 864.4[b]
AST (IU/L)	96.07 ± 33.2[a]	213.6 ± 75.1[a]	448.8 ± 84.04[b]	968.86 ± 218.1[c]
LDH (IU/L)	414.1 ± 181.3[a]	804.5 ± 82.1[b]	1077.2 ± 191.9[c]	2090.8 ± 679.7[d]
GGT (IU/L)	1.37 ± 0.51[a]	2.00 ± 0.7[a]	2.12 ± 0.99[a]	4.00 ± 2.07[b]
Col-I (ng/l)	218.1 ± 31.4[a]	339.3 ± 18.5[b]	380.8 ± 17.9[c]	488.8 ± 30.4[d]
Col-III (ng/l)	7.96 ± 1.7[a]	6.48 ± 0.18[b]	5.91 ± 0.24[b]	5.12 ± 0.01[c]

Data are expressed as mean ± standard deviation. N number is 8 in each group. a, b, c, d: Different lower cases in the same column represent statistically significant differences ab, ac, ad, bc, bd, and cd: $p<0.01$ or $p<0.001$ and same lower cases in the same column represent statistically not significant differences. aa, bb, and cc: $p>0.05$. ALT: alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactase dehydrogenase, GGT: Gamma-glutamyl transferase, Col-I: Type 1 collagen, Col-III:Type III collagen,

rats belonging to liver injury groups was significantly higher than the control group. Serum 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 and severe groups, there was no significant difference between the mild and moderate groups and the moderate and severe groups (Table 2).

Histopathological findings: In histopathological examination of tissue sections prepared from the livers of rats; in the control group, normal histological appearances of the portal regions and remark cords were recorded (Figure 1A). In CCL_4 applied groups, macro and microvesicular oil vacuoles (arrows and *) of different sizes in hepatocytes (oil degeneration), especially around the vena centralis, were noted in large areas in the parenchyma (Figures 1 B, C, D). In some of these hepatocytes, the core was pushed aside and was pycnotic (necrosis). Also, necrotic hepatocytes with basophilic pycnotic core and dark eosinophilic cytoplasm (arrowsheads) were seen in the parenchyma (coagulation necrosis), they were particularly more common around the vena centralis. Mononuclear cell infiltrates in the parenchyma and in the portal spaces and an increase in the number of perisinusoidal cells were noted. Fibrosis was particularly observed in the portal regions and around the vena centralis. In some regions, fibrous bridgings was detected between the portal regions, and extending from the portal regions to the vena centralis. As a result of all these lesions in the liver parenchyma, the remark cord structure of hepatocytes was distorted and the sinusoids were narrowed (dissociation) in proportion to the extent and severity of the lesions. As a result of severe vacuolar degeneration, bile pigment accumulation was observed in some hepatocytes (intrahepatic cholestasis) due to obstruction of bile flow. Similar morphological changes were observed in "Mild (M1), Moderate (M2) and Severe (S)" application groups, although different in the prevalence and severity of lesions. Scoring of the basic morphological changes (degeneration, necrosis and fibrosis) observed in the livers of the groups are shown in Table 3.

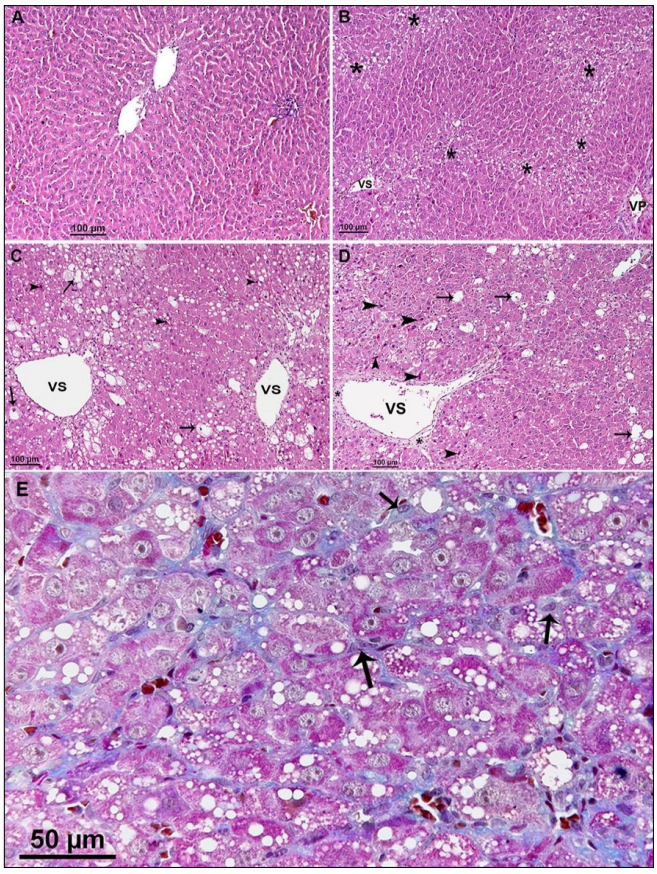

Figure 1. (A) Control group: Normal histological appearance of the liver. (B) M1 group: Mild vacuolar fat degeneration (*) more prominent around the vena centralis (VS). (C) M2 group: showing widespread macro and microvesicular fat vacuoles (arrows) of different sizes, and sporadic necrotic hepatocytes (arrowheads) in hepatocytes around the vena centralis (VS) and midzonal regions. (D) S group: Fibrosis (*) around the vena centralis (VS), necrotic changes in hepatocytes (arrowheads), diffuse macro and microvesicular oil vacuoles (arrows). H.E., Bar; 100. (E) Increase in perisinusoidal cells (arrows) and bluish colored collagen accumulation (fibrosis) in Disse intervals. Masson trichrome staining, Bar; 50.

Table 3. Effects of CCL_4 on the liver tissue of rats

Changes/ Lesions İn liver	Groups							
	Control		Mild		Moderate		Severe	
Degeneration	8/8	0	8/6	+	8/2	++++	8/5	++++
			8/2	++	8/4	++	8/2	++
					8/2	+	8/1	++
Necrosis	8/8	0	8/6	+	8/2	+++	8/5	+++
			8/2	++	8/4	++	8/2	++++
					8/2	+	8/1	++
Fibrosis	8/8	0	8/6	0	8/2	+++	8/5	++++
			8/2	+	8/4	++	8/2	+++
					8/2	+++	8/1	++

Control group, which was healthy, and it was given a regular standard pellet diet. The Mild liver injury (group 2) was injected subcutaneously with 2 ml/kg of 25% CCl_4 in paraffin oil twice a week for four weeks. Moderate liver injury (group 3), the same procedure as the mild group was applied to this group (injected subcutaneously with 2 ml/kg of 25% CCl_4 in paraffin oil twice a week for four weeks). And to make a moderate group, the procedure was followed by subcutaneous injection of 2 ml/kg of 50% CCl_4 in paraffin oil for the two weeks (6 weeks in all). Severe liver injury (group 4), which CCl_4 was administered to this group in three stages with three different doses for a total of 8 weeks.

Discussion

Today, liver diseases are one of the most common diseases globally. Hence, to assess the success and achieves early diagnosis and 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 alterations in rats with different degrees of liver injury. Carbon tetrachloride is one of the most common hepatotoxin drugs used in experimental hepatopathy studies (Eidi et al., 2015). This drug has been widely used in rodents as a model for studying and monitoring hepatic injury mechanisms (Raafat et al., 2015). 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 other researchers (Eidi et al., 2015; Raafat et al., 2015). It has been reported (Nwidu et al., 2018) that in rats induced with CCl4, RBC and Hb concentrations were decreased, and microcytic hypochromic anemia was observed. Another study stated that using CCl_4 induces leukocytosis (Asmaa, Al-Diwan, AL-Jadaan, 2018; Nwidu et al., 2018). In one research based on rats where CCl_4 was used orally, RBC, Hb, and PCV values were reported to be decreased, and all hematological parameters were affected (Mandal et al., 1998). The connection between liver-related problems and serum WBC counts has been identified in some studies. Studies have been indicated that WBC counts are related to the risk of hepatic diseases occurring. Therefore, counting of serum WBC could be useful in the process of detecting liver-related diseases (Chung et al., 2016; Jie et al., 2018). Decrease the ratio of Platelet to White Blood Cells may be an indicator of the progression of the liver disease to a worse condition, which is due to the body's comprehensive response to infection (Jie et al., 2018). Results obtained from the present study, according to Table 1, revealed that results came in line with other studies related to this topic (Chung et al., 2016; Jie et al., 2018).

Serum enzymes like ALT, AST, LDH, and ALP are frequently used parameters to examine liver dysfunction (Rahmouni et al., 2017). ALT and AST are now widely used as reliable biomarkers for the diagnosis of CCl_4-induced liver cell damage, in addition to other biochemical enzymes known as intracellular biomarkers, which are found in the bloodstream after cell membrane injury (McGill, 2016). Serum LDH was another biomarker that is not reputable to detect liver diseases; however, it could be remarkably increased due to ischemic problems, hepatic disorders related to hemolysis, and some tumors (Gitlin, Serio, 1992). The current study found that blood serum enzyme concentrations of ALT, AST, LDH, and GGT of rats with mild, moderate, and severe liver injury were higher than the control group. Also, there was a positive correlation between the increase in enzyme activity concentrations and liver damage. The highest increase in enzyme activities was detected in rats belonging to the severe group. Findings of serum enzyme activities in the current study are in line with other researchers' results and support their data (Recknagel et al., 1989; Gitlin, Serio, 1992; Althnaian, Albokhadaim, El-Bahr, 2013; McGill, 2016).

Histological survey of liver tissues has shown that CCl4 induces fibrosis, cirrhosis, and hepatocarcinoma (Althnaian, Albokhadaim, El-Bahr, 2013). Studies show that CCl4 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). Chronic hepatic injury induced by CCl_4 in rats develops hepatic fibrosis and histological and biochemical be similar to hepatic cirrhosis in both human and animals. Therefore, liver cirrhosis development in the rat model has been useful for conducting research based on liver function in human and veterinary medicine (Yanguas et al., 2016).

In the liver of CCl_4 applied rats, it was determined that degenerations in the group M1 were limited in centrilobular regions, no large macrovesicles were formed, coagulation necrosis in hepatocytes was very little, and fibrosis did not occur in most of the rats due to the low parenchymal damage. It was noted that degenerations in the group M2 also included the midzonal regions, severe and widespread macro and micro vesicular fat vacuoles were formed in hepatocytes, and hepatocellular necrosis and fibrosis were observed in most rats. It was noted that the morphological changes in the group S were similar to those in the group M2, but inflammatory, necrotic and fibrotic changes were more prominent. Differences in morphological changes between groups in rats; It is understood to be related to the density of CCl4 applied. These results were found to be similar to those of many researchers (Abdelghany et al., 2016).
Extracellular matrix consisting of connective tissue and interstitial collagen in normal liver; it is located in the portal spaces, around the central veins, and in the capsular regions. In addition, there is a thin roof consisting of extracellular matrix in which there are myofibroblastic stellate cells in the Disse gap between hepatocytes and sinusoidal endothelial cells. In cases of fibrosis and cirrhosis, primarily, excessive amounts of collagen are produced in perisinusoidal stellate cells in the Disse range. These cells, where vitamin A is normally stored, are activated during the development of fibrosis and turn into myofibroblasts. It is believed that cytokines such as TNF, IL-1 and lymphotoxins and reactive oxygen derivatives, which are thought to be produced by injured hepatocytes or by stimulated Kupffer cells and sinusoidal endothelial cells, play an important role in this transformation (Kocabayoglu, Friedman, 2013).

There are many studies on the effect of CCl4 on the making and progress of liver damage (Cheng et al., 2015; Idu, Ovuakporie-Uvo, Okojie, 2017). In this study, it was found that CCl_4-applied led to liver damage and progressed it to fibrosis (Figure1; Table 3), hence finding of this study comes in line with previous studies which was mentioned (Cheng et al., 2015; Idu, Ovuakporie-Uvo, Okojie, 2017). Liver fibrosis is an expansion changing that initially includes Collagen's raised deposition in the liver (Rojkind, 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). In this study, it was found that the amount of Collagen I increased in mild, moderate, and severe groups (9.36 ± 0.35 ng/L, 10.75 ± 0.68 ng/L, and 13.3 ± 0.78 ng/L), respectively compared to the control group ($P < 0.001$) (Table 2). While these findings indicate significant differences between all groups, it states that the highest serum level belongs to the severe group. There was a considerable difference between the control and liver injury groups and within the liver injury groups themselves. Also, it was shown that the concentration serum level of Collagen III increased in mild, moderate, and severe groups (respectively 339.3 ± 18.5 ng/L, 380.8 ± 17.9 ng/L, and 488.8 ± 30.4 ng/L) compared to the control group ($P < 0.001$). The highest amount was found to belong to the severe group (Table 2). There was a significant difference between the control group and the liver injury groups and between the liver injury groups. The current study results are consistent with previous studies mentioned (Rojkind, Perez-Tamayo, 1983; Ala-Kokko et al., 1987).

According to some articles based on research in humans and rats, it has been proven that there is a decrease in the serum level of kallistatin in liver fibrosis and liver cirrhosis (Cheng et al., 2015). In this study, serum kallistatin level in the control group was (7.96 ± 1.76 ng/ml). Compared to the control group, it was observed to decrease in the mild, moderate and severe groups (6.48 ± 0.18 ng / ml, 5.91 ± 0.024 ng/ml, and 5.12 ± 0.01 ng/ml), respectively ($P < 0.001$). Besides, the lowest amount was found to belong to the severe group. According to data, there was a significant difference between the control group and groups exposed to liver injury, and also, there was a significant difference between mild and severe groups. However, there was no difference between the mild and moderate groups, as well as between the moderate and severe groups. This data is in agreement with previous studies which was mentioned (Cheng et al., 2015; li et al., 2015; Chao, Bledsoe, Chao, 2016). In this study, it was determined that there was a negative relationship between kallistatin and other biochemical parameters; in other words, these biochemical serum parameters increased with the decrease in kallistatin serum level (Table 2). Histopathology examination of liver tissue showed no histological changes in rats belonging to the control

group. The results of this study showed that the bile duct, parenchymal cells, and Kupffer cells were normal. Hepatocyte necrosis, inflammatory cell infiltration, and bleeding foci were detected in the CCl_4-induced liver injury groups. Histological findings, changes in serum biochemical, and hematological parameters of rats with the moderate and severe liver injury caused by CCl_4 were evidence of severe cell damage in liver tissue. The histopathological results of this study support the data of other investigators (Thanh et al., 2015).

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 by different CCl_4 doses. This study demonstrates that serum kallistatin amounts are markedly lower in rats belonging to study groups (various degrees of liver injury caused by CCl_4) than healthy (control) group. It also indicates that as the intensity of hepatic injury develops, the serum kallistatin levels decrease. This study's importance is to evaluate the level changes of serum kallistatin and other biochemical parameters in rats with varying degrees of liver injury. This study aimed to evaluate the level of serum kallistatin and other biochemical parameters by inducing varying degrees of liver damage in rats by CCl_4. Despite the liver, damage to other organs such as the kidneys and lungs can also cause changes in kallistatin serum level. The protective role of kallistatin has been described in many studies (Chao, Bledsoe, Chao, 2016; Chao, Li, Chao, 2017; Wang et al., 2020). Administration of kallistatin has been shown to reduce liver disease progression (Cheng et al., 2015). According to the present study results, it is thought that measuring serum kallistatin level and other parameters could be useful in evaluating and interpreting the response to treatment in liver-related disorders. It can also be considered as a potential biomarker to detect the progression of liver injuries. Moreover, kallistatin may be promising as a candidate drug for the prevention and treatment of liver disease and may also be a treatment of other inflammatory organs discussed previously, and may add clues for kallistatin therapy/ prevention approaches.

Conclusions

As a result, in this study, it was found that decreased serum kallistatin levels were associated with liver injury. This finding can lead us to introduce kallistatin as a potential biomarker that can help for more accurate detection besides other diagnostic methods of liver disorders. As discussed earlier, kallistatin may aid early diagnosis and be considered a promising biomarker for liver disease, but more research is needed on this topic.

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