EFFECT OF *Laurus nobilis* EXTRACT ON THE FUNCTIONING OF LIVER AGAINST CCL\(_4\) INDUCED TOXICITY

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**KEYWORDS**

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**ABSTRACT**

In present study effect of laurel leaf extract (*Laurus nobilis*) on biochemical parameters and histomorphology of rat liver induced by toxic damage of CCL\(_4\) was studied. Introduction of CCL\(_4\) in animals was always carried out in fatal to 36 hours. A single injection of the *L. nobilis* extract simultaneously with CCL\(_4\) leads to 100% survivability. To evaluate the degree of hepatocyte damage in experimental modeling hepatitis de Ritis index was used. It has been reported that intraperitoneal injection of a lethal dose of CCL\(_4\) activates hepatocyte damage in the key markers, such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, \(\gamma\) -glutamyltransferase, bilirubin, urea, albumin, glucose, cholesterol, triacylglycerides. Simultaneous treatment of CCL\(_4\) with *Laurus nobilis* leaves extract prevented death and exhibit a normalizing effect on the main indicators of liver damage and index de-Ritis. Result of the present study revealed that *L. nobilis* extract have capacity to manage metabolic and histological abnormalities of hepatocytes toxic damage induced by CCL\(_4\).

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1 Introduction

The problem associated with the liver disorder need immediate attention and necessary treatment on priority basis. Despite the large arsenal used hepatoprotectors, modern medicines are not always helpful to achieve an increase in regenerative activity and prevent the development of fibrosis and cirrhosis. In recent years expanded the search of new drugs including plant origin which having a wide spectrum of pharmacological activity and economic accessibility (Voronina et al., 1996). Among them laurel (L. nobilis) has been positively evaluated by various researchers and explored wide range of pharmacological activities. Antioxidant and wound-healing effect of the ethanolic extract of L. nobilis has been well reported by the same authors in their previous studies (Vardapetyan et al., 2013). Furthermore, diuretic, antimicrobial, better appetite and digestion, used in the treatment of gastrointestinal diseases, rheumatism, migraines like various pharmacological activities have been also reported by various researchers (Buto et al., 1990; Santos & Rao, 2000). The volatiles oils of laurel suppress the development of the tuberculosis and activate the overall immune system. Leaves of the laurel are rich in phytoncides, many needed trace element and tannins which help in the riding of body toxins (Ruchkyan et al., 2013). L. nobilis is also used for preventing and treating of Type II of diabetes because it reduces the level of glucose in the blood serum (Khan et al., 2009). Furthermore it helps in reducing the level of total cholesterol and LDL-cholesterol simultaneously enhancing HDL-cholesterol (Khan et al., 2009).

One of the classic models of toxic liver damage is the intraperitoneal administration of CCl₄ (Fischer-Nielsen et al., 1991). Depending on the volume of damage (cytolysis) liver cells released equal amounts of enzyme-markers related to hepatocyte damage viz cytoplasmic alanine and aspartate aminotransferase (ALT and AST) etc into the bloodstream (Singh et al., 2011). Specific determinants hepatocyte cytolysis is ALT activity, observed as some increase AST (Yakubu et al., 2005).

ALT is an endogenous enzyme of transferases group and widely used in the diagnosis of liver damage, it has aminotransferase subgroup which catalyzing a reaction between L-alanine and 2-oxoglutarate (Zamin et al., 2002).

AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate with the oxaloacetate and L-glutamate formation. Then, oxaloacetate reacts with NADH with the formation of NAD⁺. Unlike ALT presence of the AST was reported from many tissues like myocardium, liver, skeletal muscle, kidney, pancreas, brain tissues, spleen, being less than a characteristic of liver function. Furthermore, AST is a biochemical marker which used for diagnosis of myocardial damage (Zamin et al., 2002). From the ratio of AST / ALT (coefficient De-Ritis) severity of liver damage can be judged (Gogoi et al., 2012; Roitberg & Strutynski, 1999).

Alkaline phosphatase (ALP) phosphohydrolasa of orthophosphoric acid monoesters presents in all human organs but its higher activity was reported in the liver, osteoblast cells, the placenta and intestinal epithelium. The liver ALP localizes in endothelial cells of the central and portal veins as in the sinusoids and in the bile canaliculi. Increases in the level and activity of the ALP can be observed most often in cholestasis and in some diseases of the bones (Shipman et al., 2013; Zamin et al., 2002).

γ - glutamyl transferase (GGT) - catalyzes the transfer of γ-glutamyl on amino acid, peptide or other molecule with the amino acid transport across the cell membrane. GGT has been considered as the most sensitive indicators for pathology of the biliary tract (Liu et al., 1998). Concentration of GGT in blood only increased in cases of malfunctioning of liver, biliary tract and pancreas (Lee et al., 2004). GGT is mainly contained in the cell membrane with high secretion or adsorption ability. At elimination cholestasis GGT activity is normalized faster than other enzymes. Decreased activity of GGT in cirrhosis is a reliable prognostic sign (Gogoi et al., 2012). Determination of GGT is also a sensitive test for the determination of hepatotoxic substances. So, poor liver toxicity, alcohol, drugs and certain medications are accompanied by moderate increase GGT activity in plasma. A more pronounced increase enzyme activity associated with intrahepatic or extrahepatic obstruction, tumor metastases to the liver.

TAG and cholesterol levels in blood are one of the most important determinants of lipid metabolism, that significantly increase at hyperlipidemia, developed on the base of two types of diabetes, nephrotic syndrome, chronic renal failure, obesity, acute pancreatitis, alcoholism, gout etc. In hepatocytes, phospholipid synthesis is inhibited, therefore it accumulated neutral lipid and developed fatty liver, triglycerides which cover up to 80% of the liver weight (Goldstein et al., 2006; Hashimoto et al., 2009). Hepatocyte’s pathogenesis at liver are also inhibited esterification processes and cholesterol synthesis so accumulated acetic acid is the substrate for its formation. In large quantities acetic acid manifests a cytoxic effect. Cholesterol is required for the synthesis of the lipid membrane structures, biliary acids (Goldstein et al., 2006). The role of bile acids in cholesterol metabolism is significant, so varieties of metabolic disorders of bile acids are accompanied by serious violations of cholesterol metabolism. In the blood hepatic failure reduced the content of cholesterol ester and increased the level of free cholesterol (Yatsuji et al., 2009).

Urea is the end product of protein catabolism. One of the most informative indicators used in the diagnosis of diseases the excretory system of man is the urea content in serum and urine (Arias et al., 2009). The aim of the study was to evaluate the effects of L. nobilis extracts as hepatoprotector under the cytoxic effects of CCl₄, while simultaneously research histomorphology and basic biochemical markers toxic defeat of hepatocytes.
2 Materials and Methods

2.1 The extract preparation

The extract was prepared by the 1g dried bay leaves with a homogenizer in 10 ml of 96% ethanol, standardized for flavonoid content (Vardapetyan et al., 2013) and injected 0.2 ml per 100 g rat mass. Extract for injection contains 0.8 mg of flavonoids.

2.2 Experimental Animal

Male Wistar rats (170-200g) were obtained from L.A. Orbeli Institute of Physiology NAS RA and housed in standard environmental conditions with temperature (22±3°C), humidity (60±5%) and a 12 h light/dark cycle with free access to food and water. These animals were physically active and consuming food and water in a regular way. The experimental protocol has been approved according to the local animal protection guideline of L.A. Orbeli Institute of Physiology.

All the animals were divided into 3 groups with 5 animals each group. Animals of I group were served as a untreated control. Toxic liver injury was modeled by CCl4 intraperitoneal injection in a dose of 0.2 ml per 100 g rats mass. Animals of the II group were injected intraperitoneally with 0.2 ml of CCl4 and ethanol per 100g mass, and the III group were injected intraperitoneally with 0.2ml on CCl4 and ethanolic extract of laurel leaves. The animals of the I and II groups were decapitated after 36 hours, while the animal of group III - were decapitated after 36, 240 and 480 hours, respectively. The rats were sacrificed under light ether narcosis followed by decapitation obtain biomaterials (blood, liver, kidneys) for research. Blood sample was collected from the portal vein on 0.109M citrate anticoagulant, plasma supernatant was decanted following a 15 min. centrifugation at 2.500 g.

2.3. Determination of Alanine transaminase

Determination of enzymes activity in rats blood plasma was carried out by automated analyzer (Cobas Integra 400 Plus, USA) and non-enzymatic parameters (Cobas Integra e-411, USA ) as described by the Hollman & Katan (1999). The quantitative determination of ALT was carried out by the Warburg method with pyridoxal-5-phosphate at 340 nm according to the International Federation of Clinical Chemistry (IFCC) protocol (Bergmeyer et al., 1986).

2.4 Aspartate transaminase determination

The levels of AST in rats blood plasma was determined by the Warburg method in accordance with the IFCC protocol without the pyridoxal-5-phosphate (Bergmeyer et al., 1986). AST catalyzes the transfer of an amino group between L- aspartate and 2-oxoglutarate with formation of oxaloacetate and L-glutamate. Then this oxaloacetate reacts with NADH to form NAD+. The speed of the NADH oxidation is proportional to the AST activity and was determined by spectrometric evaluation at 340 nm. Hepatocyte damage was evaluated by De-Rytis index (Singh et al., 2011; Roitberg & Strutynski, 1999), which represents the ratio of serum AST/ALT and commonly used to assess the functional status of the liver and cardiovascular system.

2.5 Alcaline phosphatase determination

ALP was measured by Bessey-Lowry-Brock method in accordance with the protocol DGKCC, based on the p-nitrophenylphosphate enzymatic hydrolysis (Severin, 2003).

2.6 γ-glutamyl transferase determination

GGT activity was determined by measuring the rate of enzymatic reaction of gamma-glutamyl group from a synthetic chromogenic donor substrate to the acceptor substrate in accordance with the protocol Szasz. Depending on the rate of cleavage of the substrate to form p - nitroaniline, color intensity is proportional to the enzyme activity in the sample is determined GGT activity at 405 nm (Farrance et al., 1975).

2.7 The glucose concentration measuring

The glucose concentration was determined by the glucose oxidase-peroxidase method with colorimetric completion by Trinderre (Liu et al., 1998).

2.8 Albumin concentration measuring

Albumin concentration was measured by using bromocresol green ("Albumin-Nov" from the OAC "Vector-Best") (Tvorogova, 2002).

2.9 Bilirubin determination

Bilirubin was determined by the color intensity change of the final product- azo pigment using a set of "Bilirubin Novo-A (conjugated)" company JSC "Vector-Best" (Chirikin, 2002).

2.10 Cholesterol determination

Cholesterol was determined using a reagents of "kit for cholesterol," the company "Biostart." Method is based on cholesterol esters enzymatic hydrolysis (in several steps) to form a colored product. Number of formed product is proportional to the cholesterol content (Maggi et al., 1996; Hyder et al., 2013).

2.11 TAG determination

Now a days determination of TAG is becoming more widely used for the diagnosis of various diseases and monitoring the effectiveness of the treatment. TAG was determined by a colorimetric enzymatic method at 546 nm (McGowan et al., 1983).
2.12 The urea measuring

The urea was determined using reagents kit “Novokarb” OAC “Vector-Best”. The principle of the urea measuring is based on the change in the intensity of staining ammonia compounds with sodium salicylate and sodium hypochlorite, which is directly proportional to the concentration of urea in the sample (Menshikov, 1987).

2.13 Histochemical assay

Hemorrhagic status of experimental animal’s liver was assessed by histochemical method using a digital microscope (Intel Play QX3). Livers were isolated, washed with physiological saline and fixed in 5% neutral formalin solution for 24 hours. Liver fresh frozen sections were prepared 150 micrometers thick, which were incubated in the mixture prepared according to method (Chilingarian, 1986).

2.14 Statistical analysis

Experimental results were expressed as means ± SD. All measurements were conducted three to five times. The data were analyzed by a one-way analysis of variance (ANOVA) and the value of p < 0.05 was considered as significant.

3 Results and Discussion

3.1 Biochemical parameters

Administration of CCl₄ to the animals led to a 100% lethal in 36 hours. A single injection of the extract L. nobilis together with CCl₄ leads to 100% survivability. Therefore, the effect of this extract on the main indicators of liver injury was investigated in the present study. In the biochemical analysis of blood with suspected liver pathogenesis; it is necessary to determine the various parameters like ALT, ACT, AP, GGT, bilirubin, albumin etc (Hyder et al., 2013). With this respect in present study the activity of ALT, AST, alkaline phosphatase, GGT, and levels of glucose, albumin, bilirubin, cholesterol, triglycerides, urea in blood plasma of all three groups of rats were determined. The results of the biochemical analyzes are summarized in the Table 1.

As shown in the Table under the influence of CCl₄ a significant increases was observed in the activity of ALT and AST, which is the major pathological syndromes of liver damage and characteristics of cytolysis. ALT is synthesized intracellularly in almost all tissues of the body, but most of its amount found in the liver, the amount of this enzyme found in small amount into the blood stream. If the damage of liver occurred this enzyme released into the blood stream and as a result of destruction of these cells, this increased level can be determined by laboratory methods. AST is also a biochemical marker for the diagnosis of myocardial injury. Increased AST and ALT in the blood plasma of experimental animals may indicate damaging effect of the CCl₄ action on hepatocytes and myocardial cells (Zamin et al., 2002; Singh et al., 2011; Roitberg & Strutynski, 1999).

Maximal activity of ALP have been reported in control animals plasma, which has undergone almost two-fold increase in the introduction of CCl₄. Pathological increase in the activity of ALP is observed more often in cholestasis and the other of pathological syndromes of liver damage and bone disorder (Shipman et al., 2013; Zamin et al., 2002).

Table 1 Basic biochemical parameters in blood plasma of rats in norm, after treatment CCL₄ and Laurel leaves extract.

|                      | Control | CCL₄ | CCL₄ + L. nobilis extract |
|----------------------|---------|------|--------------------------|
|                      |         | 36 h | 240 h | 480 h                   |
| ALT (U/l)            | 58.6±3.9| 120.4±11.3 | 103.4±11.7 | 84.4±5.5 | 79.0±5.4 |
| ACT (U/l)            | 196.6±6.0| 313.6±29.9 | 285±27.4 | 282.4±8.4 | 271±11.4 |
| AP (U/l)             | 374±18.4| 651.6±27.5 | 541.7±49.3 | 420.3±26.7 | 395.1±25.2 |
| GGT (U/l)            | 2.2±0.61| 3.4±0.81 | 3.2±0.75 | 2.9±0.35 | 2.6±0.31 |
| Glucose (mM/l)       | 7.7±0.4 | 5±0.3 | 5.1±0.3 | 5.2±0.5 | 6.7±0.4 |
| Albumin (g/l)        | 69.5±0.7 | 47.3±11.3 | 51.5±5.7 | 55.7±4.4 | 57.7±4.1 |
| Bilirubin (µM/l)     | 4.0±0.6 | 6.2±2.3 | 6.9±1.4 | 11.7±1.3 | 9.3±1.2 |
| Cholesterol (mM/l)   | 1.4±0.1 | 1.8±0.7 | 1.1±0.5 | 0.5±0.3 | 0.7±0.1 |
| TAG (mM/l)           | 1.6±0.06 | 1.7±0.09 | 1.2±0.04 | 0.7±0.03 | 0.6±0.04 |
| Urea (mM/l)          | 4.6±0.34 | 5.4±0.41 | 4.4±0.19 | 3.5±0.19 | 3.3±0.19 |

Values are the mean of four independent experiments (mean ± SD, p<0.05).
GGT activity in plasma was low compared to other tissues but it increases 1.5 times when exposed to CCl₄. The most frequent cause of increasing GGT activity in plasma is cholestasis in chronic hepatitis (Lee et al., 2004; Gogoi et al., 2012). As shown in the table, an increase in the level of conjugated (direct) bilirubin is a characteristic of cholestasis, which is based on the return of bilirubin in the blood after its conjugation and violation of free bilirubin capture by hepatocytes. Such changes are due to disturbance of hepatocytes themselves.

In the IInd group animals blood plasma observed a significant decrease in the level of albumin, the main protein of plasma synthesized only in the liver, indicating a lack of its synthetic function. The half-life of albumin is in the range 7-26 days, and therefore change its level in the monitoring period (20 days) may be a reflection of the reduced stock of albumin in plasma, and may in the normal plasma albumin reserve also be pronounced dilution effect. Decreasing the amount of albumin in plasma is accompanied by a decrease in plasma oncotic pressure; increased levels of free amino acids, amino nitrogen, development aminoaciduria (hepatic productional hyperasotemia). In the CCl₄ treated animals plasma observed some increases of urea synthesized in the liver at neutralization of ammonia formed in the of amino acids deamination. The concentration of urea in plasma depends on its synthesis rate, glomerular filtration rate and renal perfusion. Urea is the osmotically active substance, so it accumulation leads to tissue edema of parehimatoznyh organs, infarction, central nervous system, subcutaneous tissue (Arias et al., 2009). The concentration of urea in plasma is often used as an indicator of renal glomerular function. Concentration of Urea in blood plasma increased with dehydration due to enhanced passive reabsorption in the renal tubules and extrarenal azotemia retention: (impairment of renal hemodynamics). IInd group animals always had one kidney hypertrophy (Figure. 1), which could lead to increased levels of plasma urea.
On the contrary glucose level decreased by 1.5 times in the II\textsuperscript{nd} group rat plasma that constitutes a violation of carbohydrate metabolism in the liver pathology due to the depletion of glucose in the liver depot, lipid metabolism, reducing the body's ability to maintain normal blood glucose level (Brunt & Tiniakos, 2010). Liver failure is also characterized by disturbance of glucose oxidation, gluconeogenesis, the conversion of galactose to glucose and fructose (Gogoi et al., 2012). The reasons may be as malnutrition, as observed in animal’s apathy and lack of appetite for 2-3 days and/or toxicity of CCl\textsubscript{4}.

In the blood plasma of II\textsuperscript{nd} group animals an increase in the amount of cholesterol (1.3-fold) and TAG (1.1-fold) as compared to the intact animals was observed. Cholesterol main metabolic pathways in hepatocytes include cholesterol de novo synthesis and cholesterol uptake in the form of LDL and chylomicron remnants (Enjoji et al., 2012), cholesterol excretion into the blood in the form of VLDL and uptake through bile via ABCG5/G8 and NPC1L1, respectively (Nakamuta et al., 2009). The maximum proportion of cholesterol is used for bile acid synthesis. Under normal conditions, these pathways interact with each other. SREBPs act as regulators of hepatic cholesterol levels and activate genes involved in the synthesis of cholesterol and free fatty acids carried out on the basis of feedback system regulation (Enjoji et al., 2012). However during liver pathogenesis these systems are highly disorganized and in the context of liver damage the regulatory loop of SREBP is disturbed, regardless of the intracellular levels of cholesterol and/or fatty acids (Donohue, 2007), and despite excess cholesterol accumulation in hepatocytes, de novo cholesterol synthesis remained greatly enhanced even though SREBP-2 expression while it uptake suppressed because of markedly downregulated expression of LDL receptor (Enjoji et al., 2012). Excretion of Cholesterol can be enhanced by overexpression of ABCG5/G8, apolipoprotein B, and microsomal triglyceride transfer protein. This is because excess levels of cholesterol and its oxysterol metabolites, which are agonists for liver X receptor-\(\alpha\) (LXR\(\alpha\)) (Zelcer & Tontonoz, 2006), cause excess fatty acid synthesis by activating the LXR\(\alpha\)- LXR\(\alpha\) expression upregulated in the liver (Sugimoto et al., 2002).

In the III\textsuperscript{rd} group animals normalization tendency of all the above mentioned enzymes, indicating hepatoprotective properties of the \(L.\ nobilis\) extract. This is probably due to the presence and combined action of the extract phytocomponents which have flavonoid nonflavonoid origin such as, terpenes and terpenoids possessing antioxidative and antimicrobial activities (Silva & Fernandes, 2010., Santos & Rao 2000., Vardapetyan et al., 2013).

In animals group III\textsuperscript{rd} a reduction in plasma urea content as comparison with control group was reported, which usually occurs in violation of liver synthetic function. It is observed also cholesterol and TAG levels decrease in compare with control rats that certify possibility of liver damage in transition chronic form. Causes accumulation of TAG and cholesterol in
liver at CCL$_4$ induced pathology may be a violation of protein-synthetic function of the hepatocytes, inhibition of the formation of lipoprotein complexes, enters the blood as a result of all SREBPs pathways disruption both disruption of biological oxidation systems and the associated reduction in the catabolism of lipids in hepatocytes. Results of the present study are in agreement with Khan et al. (2009) those have reported that consumption of bay leaf powder reduced serum glucose significantly, TAG, total cholesterol and LDL cholesterol decrease after 30 days (Khan et al., 2009).

Using the leaf extract of $L$. nobilis normalization the level of basic biochemical markers in the blood of experimental animals. In particular, reduction in the concentration of ALT, AST, ALP and GGT was reported upto 30, 21, 68, and 35%, respectively. All the changes in the level of ALT, AST, APh, GGT (%) were represented in the figure 2.

To assess the credibility of the degree of hepatocytes damage in the modeling of experimental hepatitis (CCL$_4$) the de- Ritis index (AST / ALT) used (Menshikov 1987a; Zamin et al., 2002). Deviation of the index in the direction of decreasing evidence of damaged hepatocytes, while the increase in this indicator shows a damaged heart tissue. The value of de- Ritis index in the control group was 3.4 while this value was 2.6 in the II$_{rd}$ group, which clearly shows the deviation from the norm, characterized by liver damage. Index De-Ritis for the group III$_{rd}$ rats was equal to 2.8; 3.35 and 3.43 for subgroups of animals decapitated after 36, 240 and 480 hours, respectively. Thus introduction of bay leaf extract led to normalization tendency of the activity of ALT and AST expressed by De-Ritis- coefficient which can be placed in the following decreasing order CCL$_4$> 36 hours> 240 hours> 480 hours after injection> norm.

3.2 Histochemical assay

Under the influence of CCl$_4$ microscopically normal structure of the liver are violated. In II$_{nd}$ group animals the signs of diffuse liver disease with extensive necrosis of hepatocytes, perivascular and pericellular edema are determined (Figure. 1 B; Figure. 3 B). Representative images of hematoxylin staining to visualize architecture of the liver and hepatocytes on sections of the liver section (40-50 microns) from control rats and treated with CCl$_4$ with/without $L$. nobilis extract are in Figure.3. It is also noted that diffuse lesion of hepatocytes with more severe changes in pericapillary areas. Often noticeable discomplexation of hepatic beams. Degeneration and necrosis of hepatocytes accompanied by changes in cell membrane permeability. Hepatocyte necrosis developed, captures individual cells or small groups of cells frequently. In the foci of necrosis observed inflammatory infiltration, clearly visible in the central part of the hepatic beams.

Figure 3 Photomicrographs of rat liver similar structures sections of the: I-st group A- (control), II-nd group, B - (CCL$_4$), III-rd group C - (240h), D - (480h) All photomicrographs were taken at a magnification of 200x. The scale bar represents 50μM. Data are means ± SD of five independent experiments, p<0.05).
In III\textsuperscript{rd} group animals throughout the experiment showed improvement in the preservation of hepatocytes both in the 10 day experiment, and at day 20 (Figure 1 C; Figure 3 C & D). In primary liver tissue the processes of neutralization and reduction in the density of necrotic foci was observed. Much of the tissue did not differ from the normal liver tissue. There were no signs of edema and tissue infiltration. Thus, the histological study allows ascertaining the presence angioprotective action of the extract on the capillary bed of the rat liver and vascular protective effect on liver tissue by CCl\textsubscript{4} cytotoxic liver damage.

Thus data of morphological studies correlated with changes in the main markers of liver damage. The III\textsuperscript{rd} group animals have signs of diffuse liver disease with extensive necrosis of hepatocytes, accompanied by a decrease in the value of the index De Ritis, compared with intact animals. Also, there is a activation of ALP, GGT, bilirubin, and increasing the amount of urea, and reducing the level of albumin and glucose. All above mentioned shows the development of acute hepatitis in animals. Data of histological studies allow ascertaining the presence angioprotective action of laurel leaf extract on vascular capillary bed of the rat liver and vascular protective effect on hepatocytes at lower density foci of necrosis. This was accompanied by normalization of liver function, reflected in the above status determinants of liver.

CCl\textsubscript{4} administration to the animals led to a 100% lethal to 36 hours. A single injection of the extract \textit{L. nobilis} simultaneous with CCl\textsubscript{4} leads to 100% survivability. There are “two-hit theory”, adopted to explain ultimately cause liver damage (James & Day, 1998) according to which the one of the hit involves oxidative stress, mitochondrial dysfunction, and inflammation. The protective effect of \textit{L.nobilis} extract administration leads to prevent the inflammation and necrosis progression rather due to efficient antioxidants such as flavonoids with free radical scavenging activity and reported to play an important role in augmenting the wound-healing process. Its also bind adhesins that have been termed as the most important determinant of pathogenicity (Silva & Fernandes, 2010; Croxen & Finlay 2010; Vattem et al., 2007). The protective effect of terpenes and terpenoids can be because of the presence of \textit{p}-cymene - a precursor of carvacrol 1,8-cineole, tujen, a-pinene, o-kimen, p -kimen, \textit{γ}-terpinene and \textit{β}-phellandrene (Ultee et al., 2002; Braga et al., 2008).

The presence of the high contents of eugenol, methyl eugenol and fatty acid methyl esters together with other active components was reported (Marzouki et al., 2009) and it could contribute to its overall antioxidant and antibacterial activity (Croxen & Finlay, 2010) are also known to promote the wound-healing processes (Ultee et al., 2002; Vardapetyan et al. 2014). Along with the normalization of the liver to the 20th day there is a loosening of liver tissue (Figure 1 (C)) and signs of expressed lipidation, accompanied by a decrease in the level of urea, albumin, cholesterol and TAG below those of the intact group that sertify possibility of liver damage in transition chronic form. Accumulation of TAG and cholesterol in liver can be due to a violation of protein-synthetic function of the hepatocytes, inhibition of the formation of lipoprotein complexes, enters the blood as a result of all SREBPs pathways and biological oxidation systems disruption and the associated reduction in the catabolism of lipids in hepatocytes. Our results agree with consumption of bay leaf powder reduced serum glucose significantly, TAG, total cholesterol and LDL cholesterol decrease after 30 days (Khan et al., 2009). It is possible that the main route of metabolism of cholesterol becomes formation of bile acids, which is subject to further studies.

**Conflict of interest**

The authors declare no conflict of interest.

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