International Journal of Virology and Molecular Biology 2025, 14(6): 156-164
DOI: 10.5923/j.ijvmb.20251406.13

Hepatoprotective Activity of Isoquinoline Alkaloids (F-5, F-24), and Their Derivatives (KV-6, KV-8) in CCl_4-Induced Hepatotoxity

Abdubokiev R. Abduvali[1,*], Khushmatov S. Shunkor[2], Komilov J. Esoxon[3], Makhmudov Lazizbek Umarovich[4], Zhurakulov N. Sherzod[5]

[1]Namangan State University, Republic of Uzbekistan
[2]Ministry of Higher Education, Science and Innovation of the Republic of Uzbekistan
[3]Institute of Biophysics and Biochemistry of National University of Uzbekistan, Tashkent, Republic of Uzbekistan
[4]Institute of Bioorganic Chemistry, Uzbek Academy of Sciences, Tashkent, Republic of Uzbekistan
[5]Institute of the Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent, Republic of Uzbekistan

Abstract Experiments have shown that under the conditions of experimental CCl4 toxic hepatitis, significant changes in the external morphoanatomical structure of the mouse liver are observed. In the experimental group, under the influence of isoquinoline alkaloids (F-5, F-24) and their flavonoid conjugates (KV-6, KV-8), positive dynamics were observed, the "liver index" values were optimized, and survival rates significantly increased.

Keywords Isoquinoline alkaloids, CCl_4-induced hepatotoxity, Hepatoprotective effect

1. Introduction

In the body, the liver plays an important role in normal physiological and pathophysiological processes of detoxification, carbohydrate, protein and lipid metabolism, and maintaining the functional homeostasis of the endocrine and immune systems. In turn, hepatoprotection is considered to be of great importance for the functioning of the human body as a whole and for its health [1,2,3,4,5].

It is noted that the number of liver diseases worldwide is dynamically increasing under the influence of various exogenous/endogenous factors, resulting in 2 million people dying from these diseases annually. In turn, the development of effective therapeutic approaches and drugs for hepatoprotection is of current importance [6,7,8,9,10].

It is also noted that many pharmacological drugs currently used in clinical practice have a negative, toxic effect on liver function, and therefore the creation of effective hepatoprotective drugs is relevant [11,12,13,14,15].

It has been confirmed that significant morphostructural changes in hepatocytes occur in hepatopathologies [16,17, 18,19,20].

The aim of this study was to characterize the hepatoprotective activity of isoquinoline alkaloids (F-5, F-24) and their derivatives (KV-6, KV-8) in experimental toxic hepatitis caused by CCl_4.

* Corresponding author:
Abduvaliabduboqiyev38@gmail.com (Abdubokiev R. Abduvali)
Received: Oct. 7, 2025; Accepted: Nov. 3, 2025; Published: Dec. 30, 2025
Published online at http://journal.sapub.org/ijvmb

2. Materials and Methods

The experiments were conducted in 2023-2024 in the Pharmacological laboratory of the O.S.Sodikov Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan. In the experiments, white mice (♀/♂, m=20.5-26.2 g) were used as research objects, receiving standard food (water) under standard vivarium conditions (room temperature t=+20±5 ℃, relative air humidity 75±10%, light regime 12:12 h) [21].

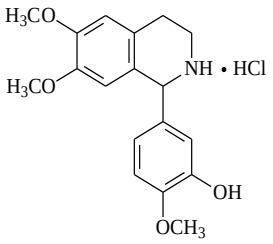
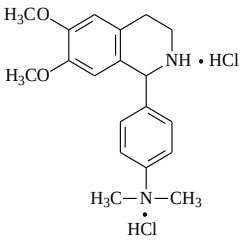
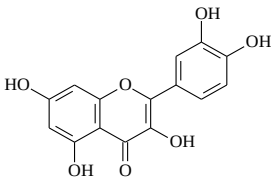
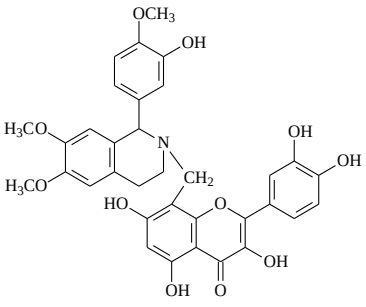
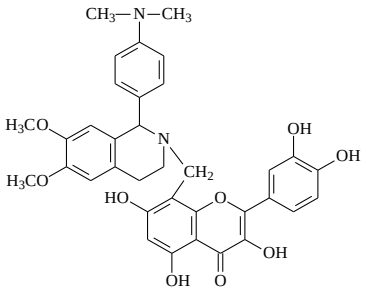
When working with experimental animals in scientific research, the requirements of the rules developed by the International Council for International Organizations of Medical Sciences (CIOMS) (1985), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), the Declaration developed by the European Union (86/609/EEC) – Regulations on bioethics of the Institute of Biophysics and Biochemistry of the National University of Uzbekistan (No. BRC/IBB; N44/2024/75-1) were observed [22,23,24]. (Table 1)

The objects of the research were isoquinoline alkaloids (F-5, F-24) and their conjugates with the flavonoid quercetin (KV-6, KV-8), synthesized by employees of the S. Yu. Yunusov Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan (Table 2) [25,26,27,28,29].

In studies, the hepatoprotective activity of isoquinoline alkaloids and their flavonoid conjugates was evaluated in a standard manner using a model of experimental toxic hepatitis in mice induced by CCl_4 [30,31,32,33,34,35].

To create an experimental model of toxic hepatitis, we used white outbred mice of both sexes (♂♀, m=20.5-26.2 g) aged 2-3 months, kept in vivarium conditions on standard food and water. In the experiments, CCl_4 solution (diluted in olive oil) was administered intraperitoneally (1 ml/kg) for 14 days (on days 1, 5, 11) [36,37,38,39,40,41,42].

Table 1. Chemical structure of isoquinoline alkaloids used in the studies – F-5, F-24, and their derivatives (KV-6, KV-8)

Code (name)	molecular weight	**Chemical structural formula**	LD_{50} (mg/kg)
F-5 (1-(3′-hydroxy-4′-methoxyphenyl)-6,7-dimethoxy -1,2,3,4-tetrahydroisoquinoline hydrochloride),	315		980
F-24 (1-(4′-dimethylaminophenyl)-6,7-dimethoxy -1,2,3,4-tetrahydroisoquinoline hydrochloride),	312		1250
Quercetin (2-(3,4-dihydroxyphenyl) -3,5,7-trihydroxy-4H-chromen-4-on),	302		
KV-6 (2-(3,4-Dihydroxyphenyl)-6-((1-(3-hydroxy-4-methoxyphenyl) -6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl) -3,5,7-trihydroxy-4H-chromen-4-on),	629		
KV-8 (2-(3,4-Dihydroxyphenyl)-6-((1-(4-dimethylaminophenyl) -6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl) -3,5,7-trihydroxy-4H-chromen-4-on),	626		

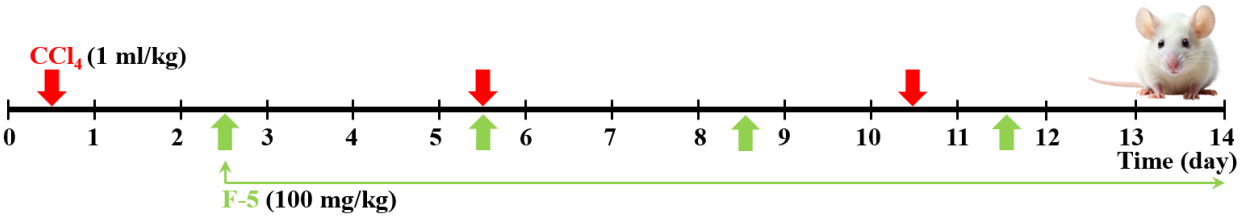


Figure 1. Experimental Design. To create an experimental model of toxic hepatitis, animals were administered CCl_4 (1 ml/kg) on days 1, 5, and 11. In an experimental model of CCl_4-toxic hepatitis, the hepatoprotective activity of isoquinoline alkaloids (F-5, F-24) and their derivatives (KV-6, KV-8) was analyzed by administering the substances to mice at a concentration of 100 mg/kg (orally mixed with olive oil (1:1)) on days 3, 6, 9, and 11 (the control group received an equivalent amount of olive oil to the experimental group) [Elshafei et al., 2013; pp.82-89]

2.1. Morpho-Anatomical Analysis of the Liver in CCl_4-Induced Hepatotoxity, and Method of Analyzing Mouse Survival

The liver of experimental animals was preserved in formalin solution (10%) for morphoanatomical analysis [Mondal et al., 2022; pp.1013-1022] (Figure 1).

In experiments analyzing the hepatoprotective activity of isoquinoline alkaloids (F-5, F-24) and their derivatives (KV-6, KV-8) in CCl4-induced hepatotoxity, the survival rate (%) of mice was estimated using the standard method – the Kaplan-Meier method [43,44,45,46,47,48].

The survival analysis curve developed by Kaplan E.L., Meyer P. (1958) was calculated based on the following formula (*2.1*) [49,50,51,52,53,54,55]:

$$S(t) = \prod_{t_i \le t}\left(1 - \frac{d_{x+t_i}}{l_{x+t_i^-}}\right) \qquad (2.1)$$

$S(t)$ – is the survival rate at a given time (t), x – is the initial value of the survival function, ($x+t_i$) – is the difference in values at the time of death, $l(x+t_i)$ – is the number of deaths that occurred before time $x+t_i$, and $dx+t_i$ – is the number of deaths observed during time $x+t_i$.

2.2. Statistical Analysis

Mathematical and statistical processing of the obtained experimental results was carried out by standard methods using specialized software packages "Microsoft Excel 2007" (Microsoft, USA), OriginPro v. 8.5 SR1 (EULA, USA). The results are presented as $M \pm m$, where M is the arithmetic mean and m is the standard error of the mean, calculated based on the results of experiments conducted with n=3-4 repetitions. Also, the level of statistical significance of the values between the experimental results and the control group was calculated based on Student's t-test and was considered statistically significant at values of $p<0.05$ and $p<0.01$. The level of reliability of the difference between the values of the two experimental groups was calculated using the Student's t-test [56,57,58,59].

3. Results and Discussion

3.1. Molecular Mechanism of Experimental Toxic Hepatitis Induced by CCl_4

Carbon tetrachloride (CCl_4) is widely used to induce a model of toxic damage to hepatocytes in the form of experimental hepatitis in laboratory animals (in vivo), which is characterized by changes in ALT, AST, malondialdehyde, glutathione, superoxide dismutase, c-Jun N-kinase (JNK), glutathione peroxidase, catalase, tumor necrosis factor-α (TNF-α), alkaline phosphatase, bilirubin, total protein, albumins, quantitative and functional indices of globulins, hepatosis, fibrosis, cirrhosis, and hepatocellular degenerative changes [60,61,62,63].

CCl_4-toxic hepatitis is an ideal experimental model for analyzing the hepatoprotective activity of various drugs in liver diseases in experimental animals [64,65,66,67].

Under the influence of CCl_4, the induction of oxidative stress in hepatocytes with the participation of TNF-α (tumor necrosis factor-α), TGF-β1 (transforming growth factor-β1) and IL-1β factors leads to inflammation, collagen accumulation and fibrosis through the activation of a cascade of degradative inflammation and the accumulation of collagen and fibrin outside the hepatocyte membrane, which leads to fibrosis/cirrhosis (Fig. 2) [68,69,70] (Fig. 3).

Activation of nuclear factor erythroid-associated factor 2 (Nrf_2), NF-κB, is believed to play an important role in the pathogenesis of experimental toxic hepatitis induced by CCl_4 in hepatocytes [Wang et al., 2022; pp.1-14].

Exposure to CCl_4 increases the concentration of cytokines (IL-1βa, IFN-γ) and chemokines (MCP-1, MIP-2β, KC), which are activated during inflammatory responses in mouse hepatocytes [71].

Moreover, experimental toxic hepatitis in rats induced by CCl_4 was associated with a sharp increase in serum ALT, AST and bilirubin levels, as well as significant pathological changes in the morphoanatomical structure of the liver [72].

Structural and functional changes in hepatocytes under the influence of CCl_4 have been described in detail by a number of researchers. It is emphasized that with the participation of cytochrome P_{450} (CYP_2E1 and other isoforms), CCl_4 forms free radicals – CCl_3•, CCl_3OO•, which in turn leads to the formation of lipid peroxidation based on a chain reaction of free radical generation, induction of apoptosis and necrosis [73].

Under the conditions of CCl_4-toxic hepatitis, activation of the TNF-a/IL-6, TGF-b1/CTGF, lipid peroxidation (LPO) cascade, and collagen accumulation in hepatocytes (The involvement of MAPK/JNK – mitogen-activated protein kinase (*Mitogen-activated protein kinase/c-Jun NH2-terminal*

kinase), Akt/NF-kB – transcription factor (*Akt regulates the transcriptional activity of nuclear factor-kB*) in the hepatotoxic process has also been confirmed), the development of fibrosis is noted [74,75].

Under conditions of experimental hepatitis induced by CCl_4, a significant increase in liver weight, pronounced morphoanatomical deformation and histopathological changes were confirmed in experimental animals [76,77].

3.2. The Influence of Isoquinoline Alkaloids (F-5, F-24) and Their Derivatives (KV-6, KV-8) on the Morphoanatomical Indices of the Liver in Experimental Toxic Hepatitis Caused by CCl_4

As a result of the experiments, it was established that under the conditions of CCl_4-experimental toxic hepatitis, pronounced changes in the external morpho-anatomical structure of the liver of mice are observed, and in the experimental group, positive changes were observed under the influence of isoquinoline alkaloids (F-5, F-24), and their derivatives (KV-6, KV-8) (Fig. 3).

Typically, the average body weight of 3-4 month old mice is 20-35 g, the "liver index" is 0.060 (liver weight 1.89 g) in males and 0.058 (liver weight 1.27 g) in females. In experimental hepatitis caused by CCl_4, animals exhibit a significant decrease in body weight, an enlarged liver and, in turn, an increase in the "liver index" value [78,79].

In the experiments, the average body weight in the control group was 23.40±0.37 g for males and 22.63±0.14 g for females, while under conditions of experimental toxic hepatitis, a decrease to 22.57±0.18 g and 21.15±0.09 g, respectively, was observed. In the physiological norm (control) state, the average liver weight of mice was m=1.05±0.01 g ($m_{min.}$=0.68±0.01 g, $m_{max.}$=1.46±0.01 g), and under experimental toxic hepatitis conditions it increased by 13.4±0.17% compared to the control (m=1.19±0.01 g, $m_{min.}$=0.94±0.01 g, $m_{max.}$=1.52±0.01 g). It was found that the liver index value averages 0.045 and 0.046 in males and females, respectively, and increases by 17.80% (0.053) and 21.74% (0.056) in toxic hepatitis compared to the control group, respectively (Table 2).

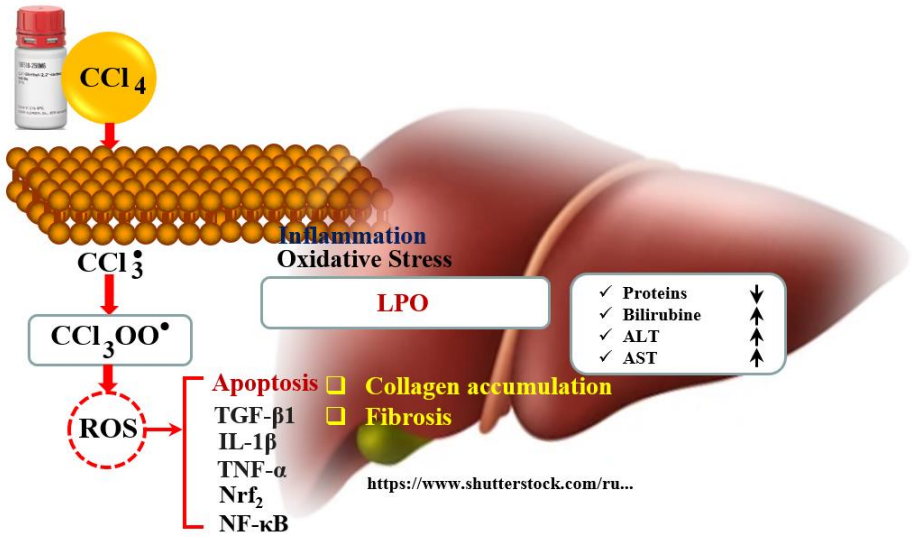


Figure 2. Molecular mechanism of experimental induction of hepatitis by CCl_4 [Ke and Lee, 2018; pp.214-223; Ma et al., 2022; pp.111-122; Bishnolia et al., 2025; pp. 1-8; Salim et al., 2025; pp.1-12]

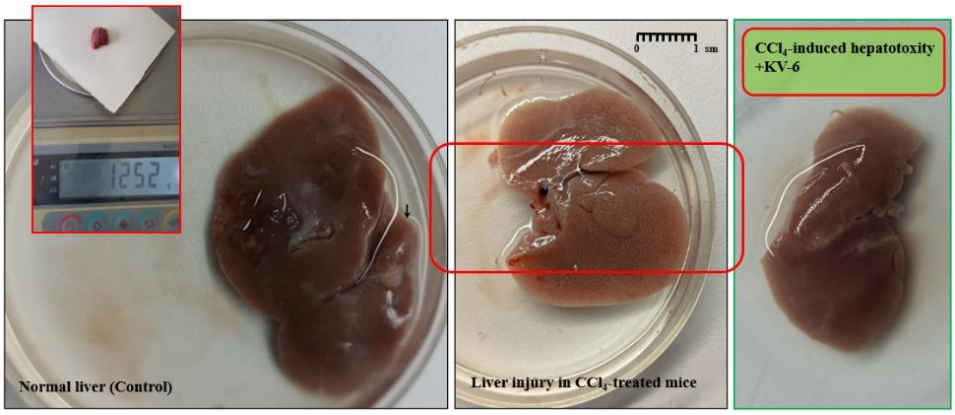


Figure 3. External morphoanatomical appearance of the liver of mice under healthy conditions (control) and toxic hepatitis CCl_4 (The effect of the KV-6)

Table 2. The effect of isoquinoline alkaloids (F-5, F-24), their derivatives (KV-6, KV-8) on the liver index in mice with CCl_4-experimental toxic hepatitis ($M \pm m$)

Index	♂/♀	Control	CCl_4	Name of the substance			
				F-5	F-24	KV-6	KV-8
Body weight (g)	♂	23.40±0.37	22.57±0.18	22.90±0.16	23.06±0,29	23.06±0.24	22.98±0.55
	♀	22.63±0.14	21.15±0.09	21.75±0.23	22.07±0,13	22.01±0.11	21.91±0.38
Liver index	♂	0.045	0.053**	0.049	0.050	0.046*	0.048*
	♀	0.046	0.056**	0.051	0.052	0.049*	0.050*

Note: *compared to control * – $p<0.05$ ** – $p<0.01$ (n=3-4).*

Table 3. Analysis of survival rates of mice under conditions of CCl_4-induced hepatotoxity

Day	Control (*Number of animals*)	CCl_4		Substances							
				F-5		F-24		KV-6		KV-8	
		l	$S_{(t)}$	l	$S_{(t)}$	l	$S_{(t)}$	l	$S_{(t)}$	l	$S_{(t)}$
4	15	6	60,0	4	73,3*	5	66,7	2	**86,7****	3	80,0**
7	15	8	46,7	6	60,0*	5	66,7*	3	**80,0****	5	66,7*
14	15	10	33,3	7	53,3**	8	46,7*	4	**73,3****	6	60,0**

Note: l – number of dead animals, $S(t)$ – survival rate (%). CCl_4 (1 ml/kg) was administered on the 1st, 5th, and 11*th* days.
The hepatoprotective activity of isoquinoline alkaloids (F-5, F-24), and their derivatives (KV-6, KV-8) was studied in CCl_4-induced hepatotoxity. The substances were administered to mice at a concentration of 100 mg/kg (orally in a mixture with olive oil) on the 3rd, 6th, 9th and 11th days. The values in the experimental groups were compared with those in the experimental model of CCl_4-induced hepatotoxity (* – $p<0.05$, ** – $p<0.01$; n=3-4).

Under the influence of F-5, the liver mass was m=1.12±0.01 g, and the "liver index" value was 0.049 and 0.051 in male and female mice, respectively. The value of this indicator was recorded at the level of 0.050 and 0.052 for F-24 (100 mg/kg) (m=1.15±0.01 g) in male and female mice, respectively. Under the influence of KV-6 (100 mg/kg), the liver mass was m=1.08±0.01 g, and the "liver index" value was 0.046 and 0.049 in male and female mice, respectively. The value of this indicator for KV-8 (m=1.10±0.01 g) was 0.048 and 0.050, respectively.

The analysis of the obtained results showed that isoquinoline alkaloids (F-5, F-24) and their flavonoid conjugates (KV-6, KV-8) have significant hepatoprotective activity under conditions of experimental toxic hepatitis caused by CCl_4, and this activity has a characteristic increase dynamic in the series F-24>F-5>KV-8>KV-6.

3.3. The Effect of Isoquinoline Alkaloids (F-5, F-24) and Their Derivatives (KV-6, KV-8) on the Survival of Mice with Experimental Toxic Hepatitis Caused by CCl_4

During the experiments, it was established that in the experimental model of CCl_4-toxic hepatitis, the S(t) value on the 4th, 7th and 14th days was 60.0, 46.7 and 33.3%, respectively, and significantly increased under the influence of isoquinoline alkaloids (F-5, F-24) and their derivatives (KV-6, KV-8) (Table 3).

It was established that the value of this indicator was 73.3, 60.0 and 53.3% for isoquinoline alkaloids for F-5, respectively, 66.7, 66.7 and 46.7% for F-24, as well as 86.7, 80.0 and 73.3% for KV-6 for derivatives (F-5, F-24), respectively, 80.0, 66.7 and 60.0% for KV-8.

This experimental model is also a convenient method for analyzing the effects of various agents on the survival rates of experimental animals in studies using the Kaplan-Meier method [80,81].

In this regard, it has been confirmed that in an experimental model of hepatitis, toxic CCl_4, the survival rate of mice is on average 70% [82,83].

The figure below shows the Kaplan-Meier estimate of the survival rate (%) of isoquinoline alkaloids (F-5, F-24), and their derivatives (KV-6, KV-8) in CCl4-induced hepatotoxity (Figure 4).

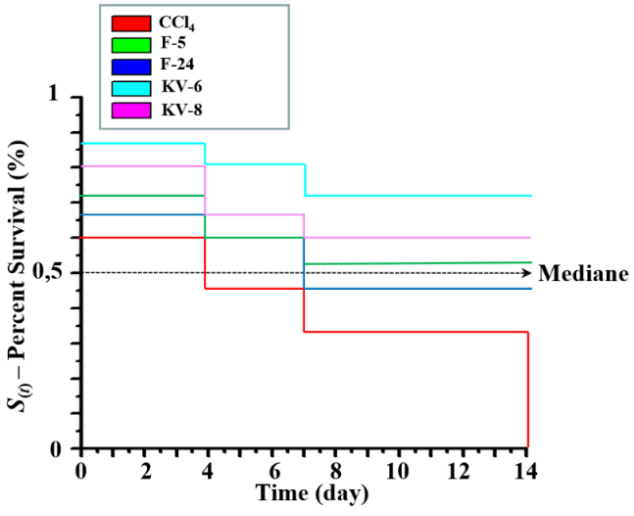


Figure 4. Kaplan-Meier survival estimate (%) of isoquinoline alkaloids (F-5, F-24), and their derivatives (KV-6, KV-8) in mice in CCl_4-induced hepatotoxity. The median value reflects the death of 50% of animals by a given point in time (t)

4. Conclusions

Thus, in an experimental model of toxic CCl_4 hepatitis, it was found that isoquinoline alkaloids (F-5, F-24), and their derivatives (KV-6, KV-8) significantly increase the survival rate of mice.

REFERENCES

[1] Abdulkhakova G.V., Komilov E.Dzh., Ergashev N.A., Asrarov M.I., Makhmudov R.R., Kenzhaeva M.F. Effect of tannins hexagalloylglucose and heptagalloylglucose on ATP-dependent potassium channels in vitro // "Universum: Chemistry and Biology" (Electronic Scientific Journal). – 2025. – №5(131). – P.59-64. (*In Russian*).

[2] Abrashova T.V., Gushchin Ya.A., Kovaleva M.A., Rybakova A.V., Selezneva A.I., Sokolova A.P., Khodko S.V. Physiological, biochemical and biometric indicators of the norm of experimental animals (Handbook) (Edited by: Prof. Makarova V.G., Makarova M.N.) // St. Petersburg: Publishing house "LEMA", 2013. – 116 p.

[3] Aditi P., Ali V., Choubey M., Tirumalasetty M.B., Pandey H., Srivastava S., Tripathi Y.B. Hepatoprotective role of Pueraria tuberosa water extract (PTWE) in CCl_4-induced liver injury through different signaling pathways // Advances in Traditional Medicine. – 2014. – P.1-16.

[4] Algefare A.I., Alfwuaires M., Famurewa A.C., Elsawy H., Sedky A. Geraniol prevents CCl_4-induced hepatotoxicity via suppression of hepatic oxidative stress, pro-inflammation and apoptosis in rats // Toxicology Reports. – 2024. – V.12. – P.128-134.

[5] Alhassan A.J., Sule M.S., Aliyu S.A., Aliyu M.D. Ideal hepatotoxicity model in rats using carbon tetrachloride (CCl_4) // Bayero Journal of Pure and Applied Sciences. – 2009. – V.2(2). – P.185-187.

[6] Ali A.A. Biochemical and Histological Study of Rat Liver Injury Induced by carbon tetrachloride (CCl_4) // Wasit Journal for Pure Sciences. – 2025. – V.4(1). – P.156-167.

[7] Anijdan S.H.M., Mahdavi S.R., Shirazi A., Zarrinfard M.A., Hajati J. Megavoltage X-ray dose enhancement with gold nanoparticles in tumor bearing mice // IJMCM. – 2013. – V.2(3). – P.118-124.

[8] Azamatov A.A., Zhurakulov S.N., Vinogradova V.I., Tursunkhodzhaeva F., Khinkar R.M., Malatani R.T., Aldurdunji M.M., Tiezzi A., Mamadalieva N.Z. Evaluation of the local anesthetic activity, acute toxicity, and structure-toxicity relationship in series of synthesized 1-aryltetrahydroisoquinoline alkaloid derivatives in vivo and in silico // Molecules 2023, 28, 477). – P.1-17.

[9] Begum R., Papia S.A., Begum M.M., Wang H., Karim R., Sultana R., Das P.R., Begum T., Islam R., Manwar N., Rahman S. Evaluation of hepatoprotective potential of polyherbal preparations in CCl_4-induced hepatotoxicity in mice // Advances in Pharmacological and Pharmaceutical Sciences. – 2022 (3169500). – P.1-9.

[10] Belyaev A. M., Mikhnin A. E., Rogachev M. V. Data preparation and survival analysis in the MedCalc and Statistica statistical software packages (Study guide for students in the system of higher and additional professional education) // St. Petersburg (N.N. Petrov National Medical Research Center of Oncology), 2022. – 56 p. (*In Russian*).

[11] Besednova N. N., Zaporozhets T. S., Kuznetsova T. A., Kryzhanovsky S. P., Kovalev N. N., Zvyagintseva T. N. Hepatoprotective effects of seaweed extracts and polysaccharides // Antibiotics and chemotherapy. – 2014. – #59(3-4). – P.30-37. (*In Russian*).

[12] Bishnolia M., Yadav P., Singh S.K., Manhar N., Rajput S., Khurana A., Bhatti K.S., Navik U. Methyl donor ameliorates CCl_4-induced liver fibrosis by inhibiting inflammation, and fibrosis through the downregulation of EGFR and DNMT-1 expression // Food and Chemical Toxicology. – 2025. – V.196(115230). – P.1-8.

[13] Bocharov E. V., Karpova R. V., Bocharova O. A., Kucheryanu V. G., Shprakh Z. S. The effect of a multiphytoadaptogen in early postnatal ontogenesis, improving the survival and somatic state of mice of a high-cancer line // Russian Journal of Biotherapeutics. – 2017. – #.16. – P.76-81. (*In Russian*).

[14] Bueverov A.O. Possibilities of treating drug-induced liver injury in conditions of the need to take hepatotoxic drugs // The attending physician. – 2009. – #.2. – P.40-42. (*In Russian*).

[15] Byun J.-H., Kim J., Choung S.-Y. Hepaprotective effect of standardized *Ecklonia stolonifera* formulation on CCl_4-induced liver injury in Sprague-Dawley rats // Biomol. Ther. – 2018. – V.26(2). – P.218-223.

[16] Chang M.-L., Yeh C.-T., Chang P.-Y., Chen J.-C. Comparison of murine cirrhosis models induced by hepatotoxin administration and common bile duct ligation // World J. Gastroenterol. – 2005. – V.11(27). – P.4167-4172.

[17] Chen P., Zhou Y.-K., Han C.-S., Chen L.-J., Wang Y.-M., Zhuang Z.-M., Lin S., Zhou Y.-H., Jiang J.-H., Yang R.-L. Stem cells from human exfoliated deciduous teeth alleviate liver cirrhosis via inhibition of gasdermin D-executed hepatocyte pyroptosis // Front. Immunol. – 2022. – V.13(860225). – P.1-12.

[18] Chen Y., Li R., Hu N., Yu C., Song H., Li Y., Dai Y., Guo Z., Li M., Zheng Y., Guo Z., Qi Y. Baihe Wuyao decoction ameliorates CCl_4-induced chronic liver injury and liver fibrosis in mice through blocking TGF-β_1/Smad2/3 signaling, anti-inflammation and anti-oxidation effects // Journal of Ethnopharmacology. – 2020. – V.263(113227). – P.1-11.

[19] Das M., Boerma M., Goree J.R., Lavoie E.G., Fausther M., Gubrij I.B., Pangle A.K., Johnson L.G., Dranoff J.A. Pathological changes in pulmonary circulation in carbon tetrachloride (CCl_4)-induced cirrhotic mice // PLoS ONE. – 2014. – V.9(4): e96043. – P.1-9.

[20] Delgado-Montemayor C., Cordero-Perez P., Salazar-Aranda R., Waksman-Minskya N. Models of hepatoprotective activity assessment // Medicina Universitaria. – 2015. – V.17(69). – P.222-228.

[21] Dong M., Yang P., Zhang X., Nie S., Sun X. Nrf_2 deficiency brings about increased sensitive to IR and 7,12-dimethylbenz(a) anthracene and leukemia predisposition // Dose-Response: An International Journal. – 2025. – V.23(2). – P.1-11.

[22] Dospekhov B.A. Methodology of field experiment (With the

basics of statistical processing of research results) // 5th edition, Moscow. – "Agropromizdat Publishing House", 1985. – P.347-415. (*In Russian*).

[23] Elshafey S.M.A., Abdelrahman A.A., Tukhbatova R.I., Ivanova E.V., Akinina E.A., Voronkova Yu.E., Bukuru L.K., Fattakhova A.N., Alimova F.K. The influence of Nigella sativa and Salvia officinalis vegetable oils on the biochemical parameters of CD-1 mice // Scientific notes of Kazan University. – 2013. – T.155(3). – P.82-89. (*In Russian*).

[24] Ernst L., Zieglowski L., Schulz M., Moss M., Meyer M.,Weiskirchen R., Palme R., Hamann M., Talbot S.R., Tolba R.H. Severity assessment in mice subjected to carbon tetrachloride // Scientific Reports. – 2020. – V.10(15790). – P.1-11.

[25] Fortea J., Fernandez-Mena C., Puerto M., Ripoll C., Almagro J., Banares J., Bellon J.M., Banares R., Vaquero J. Comparison of two protocols of carbon tetrachloride-induced cirrhosis in rats – improving yield and reproducibility // Scientific Reports. – 2018. – V.8(9163). – P.1-10.

[26] Fu X., Jiang B., Zheng B., Yan Y., Wang J., Duan Y., Li S., Yan L., Wang H., Chen B., Sang X., Ji W., Xu R.-H., Si W. Heterogenic transplantation of bone marrow-derived rhesus macaque mesenchymal stem cells ameliorates liver fibrosis induced by carbon tetrachloride in mouse // PeerJ. – 2018. – V.6(e4336). – P.1-23.

[27] Fujii T., Fuchs B.C., Yamada S., Lauwers G.Y., Kulu Y., Goodwin J.M., Lanuti M., Tanabe K.K. Research article mouse model of carbon tetrachloride induced liver fibrosis: Histopathological changes and expression of CD_{133} and epidermal growth factor // BMC Gastroenterology 2010. – V.10(79). – P.1-11.

[28] Gan C., Yuan Y., Shen H., Gao J., Kong X., Che Z., Guo Y., Wang H., Dong E., Xiao J. Liver diseases: Epidemiology, causes, trends and predictions // Signal Transduction and Targeted Therapy. – 2025. – V.10(33). – P.1-36.

[29] Ghosh A., Sil P. Protection of acetaminophen induced mitochondrial dysfunctions and hepatic necrosis via Akt-NF -kappaB pathway: Role of a novel protein // Chem. Biol. Interact. – 2009. – V.177. – P.96-106.

[30] Glanz S. Medical and Biological Statistics // Moscow. – "Praktika Publishing House". – 1999. – P.250-459. (*In Russian*).

[31] Gomes A.P., Costa B., Marques R., Nunes V., Coelho C. Kaplan-Meier survival analysis: Practical insights for clinicians // Acta Med. Port. – 2024. – V.37(4). – P.280-285.

[32] Jaeschke H., Bajt M. Intracellular signaling mechanisms of acetaminophen-induced liver cell death // Toxicol. Sci. – 2006. – V.89. – P.31-41.

[33] Kaplan E.L., Meier P. Nonparametric estimation from incomplete observations // Journal of the American Statistical Association. – 1958. – V.53(282). – P.457-481.

[34] Ke B.-J., . Lee C.-L. *Cordyceps cicadae* NTTU 868 mycelium prevents CCl_4-induced hepatic fibrosis in BALB/c mice via inhibiting the expression of pro-inflammatory and pro-fibrotic cytokines // Journal of Functional Foods. – 2018. – V.43. – P.214-223.

[35] Khadrawy S.M., Mohamed H.M., Mahmoud A.M. Mesenchymal stem cells ameliorate oxidative stress, inflammation, and hepatic fibrosis via Nrf_2/HO-1 signaling pathway in rats // Environmental Science and Pollution Research. – 2021. – V.28. – P.2019-2030.

[36] Kleinbaum D.G., Klein M. Survival analysis. A self-learning text (3 Ed.) // In "Statistics for biology and health" (Ed. Gail M., Krickeberg K., Samet J.M., Tsiatis A., Wong W.). – "Springer" (New York, Dordrecht, Heidelberg, London). – 2020. – P.1-712.

[37] Krylov D.P., Rodimova S.A., Karabut M.M., Kuznetsova D.S. Experimental Models for Studying the Structural and Functional State of the Liver during Pathology Development (Review) // Modern Technologies in Medicine. – 2023. – #15(4) – P.65-84. (*In Russian*).

[38] Lakin G.F. Biometrics // Moscow. – "Higher School Publishing House", 1990. – P.284. (*In Russian*).

[39] Lebedeva E.I. Dynamics of Structural and Functional Disorders in the Liver of Rats with Experimental Cirrhosis // Bulletin of VSMU. – 2015. – #.14(3). – P.21-31.

[40] Li J., Liu W., Zhang J., Sun C. The Role of mitochondrial quality control in liver diseases: Dawn of a therapeutic era // Int. J. Biol. Sci. – 2025. – V.10:21(4). – P.1767-1783.

[41] Li X., Chen W., Jia Z., Xiao Y., Shi A., Ma X. Mitochondrial dysfunction as a pathogenesis and therapeutic strategy for metabolic dysfunction-associated steatotic liver disease // Int. J. Mol. Sci. – 2025. – V.26(4256). – P.1-29.

[42] Lin S.-Y., Xu D., Du X.-X., Ran C.-L., Xu L., Ren S.-J., Tang Z.-T., Yin L.-Z., He C.-L., Yuan Z.-X., Fu H.-L., Zhao X.-L., Shu G. Protective effects of salidroside against carbon tetrachloride (CCl_4)-induced liver injury by initiating mitochondria to resist oxidative stress in mice // Int. J. Mol. Sci. – 2019. – V.20(3187). – P.1-14.

[43] Liu B., Fang Y., Yi R., Zhao X. Preventive effect of Blueberry extract on liver injury induced by carbon tetrachloride in mice // Foods. – 2019. – V.8(48). – P.1-13.

[44] Ma Y., Bao Y., Wu L., Ke Y., Tan L., Ren H., Song J., Zhang Q., Jin Y. IL-8 exacerbates CCl4-induced liver fibrosis in human IL-8-expressing mice via the PI3K/Akt/HIF-1α pathway // Molecular Immunology. – 2022. – V.152. – P.111-122.

[45] Makhmudov L.U., Aripov T.F., Vypova N.L., Nurbekova N.B., Yuldashev H.A., Esanov R.S., Gafurov M.B., Yakubova R.A., Tagaygalieva N.A. Antioxidant and hepatoprotective properties of complexes of monoammonium salt of glycyrrhizic acid with amino acids and phenolic compounds // Experimental and clinical pharmacology. – 2023. – #.86(3). – P.23-28. (*In Russian*).

[46] Mallaeva M.M., Mustafakulov M.A. Hepatitis is a powerful antioxidant enzyme that produces polyphenols // Computer Science and Engineering Technologies (Conference on Materials). – 2023. – #1(2). – 391-394. (*In Uzbek*).

[47] Masuda A., Nakamura T., Abe M., Iwamoto H., Sakaue T., Tanaka T., Suzuki H., Koga H., Torimura T. Promotion of liver regeneration and anti-fibrotic effects of the TGF-β receptor kinase inhibitor galunisertib in CCl_4-treated mice // International Journal of Molecular Medicine. – 2020. – V.46. – P.427-438.

[48] Miroshnikov M.V., Makarova M.N. Variability of blood biochemical parameters and the establishment of reference intervals in preclinical studies (Communication 4: Mice) //

Laboratory animals for scientific research. – 2021. – #03. – P.63-69. (*In Russian*).

[49] Mittra I., Pal K., Pancholi N., Tidke P., Siddiqui S., Rane B., D'souza J., Shaikh A., Parab S., Shinde S., Jadhav V., Shende S., Raghuram G.V. Cell-free chromatin particles released from dying host cells are global instigators of endotoxin sepsis in mice // PLoS ONE. – 2020. – V.15(3:e0229017). – P.1-22.

[50] Mondal M., Sarkar C., Saha S., Hossain N., Norouzi R., Mubarak M.S., Siyadatpanah A., Wilairatana P., Hossain R., Islam M.T., Coutinho H.D.M. Hepatoprotective activity of Andrographolide possibly through antioxidative defense mechanism in Sprague-Dawley rats // Toxicology Reports. – 2022. – V.9. – P.1013-1022.

[51] Nhung T.H., Nam N.H., Nguyen1 N.T.K., Huy L.M., Giang T.H., Nghia H., Van Thanh N. Establishment of a standardized mouse model of hepatic fibrosis for biomedical research // Biomedical Research and Therapy. – 2014. – V.1(2). – P.43-49.

[52] Nishitani M., Okada H., Nio K., Hayashi T., Terashima T., Iida N., Shimakami T., Takatori H., Honda M., Kaneko S. et al. $Mint_3$ as a molecular target activated in the early stage of hepatocarcinogenesis // Int. J. Mol. Sci. 2025. – V.26(1430). – P.1-15.

[53] Nizinski P., Krajewska A., Oniszczuk T., Polak B., Oniszczuk A. Hepatoprotective effect of kaempferol – A review // Molecules. – 2025. – V.30(1913). – P.1-25.

[54] Plokhinsky N.A. Biometrics // Moscow. – "Moscow State University Publishing House". – 1970. – P.20-367. (*In Russian*).

[55] Rafiq H., Ayaz M., Khan H.A., Iqbal M., Quraish S., Afridi S.G., Khan A., Khan B., Sher A., Siraj F., Shams S. Therapeutic potential of stem cell and melatonin on the reduction of CCl_4-induced liver fibrosis in experimental mice model // Brazilian Journal of Biology. – 2024. – V.84(e253061). – P.1-7-б.

[56] Rebrova O.Yu. Statistical Analysis of Medical Data (Application of the STATISTIKA Software Package). – Moscow. – "Media Sphere Publishing House", 2002. – P.5-312. (*In Russian*).

[57] Renner H. The limited relevance of models used for testing human hepatic diseases and their prevention (In: "Mechanisms of hepatocyte injury and death" Keppler E. et al. (Eds.) // MTP Press Ltd., Lancaster, 1985. – P.311-320.

[58] Reynolds E.S. Liver parenchymal cell injury. I. Initial alterations of the cell following poisoning with carbon tetrachloride // J. Cell Biol. – 1963. – V.19. – P.139-157.

[59] Richards S.J., Macdonald A.S. On contemporary mortality models (For actuarial use) // Ainslie Place, Edinburgh, 2024. – P.80.

[60] Ruz-Maldonado I., Gonzalez J.T., Zhang H., Sun J., Bort A., Kabir I., Kibbey R.G., Suarez Y., Greif D.M., C.Fernandez-Hernando. Heterogeneity of hepatocyte dynamics restores liver architecture after chemical, physical or viral damage // Nature Communications. – 2024. – V.15(1247). – P.1-24.

[61] Salim N.S., Abo El-Maati M.F., Abdelnour S.A., Abdel-Alim M.E. Hepatoprotective activity of *Taraxacum officinale* extract against CCl_4-induced liver injury in rats // Food Bioscience. – 2025. – V.68(106708). – P.1-12.

[62] Scholten D., Trebicka J., Liedtke C., Weiskirchen R. The carbon tetrachloride model in mice // Laboratory Animals. – 2015. – V.49(S1). – P.4-11.

[63] Sharashova E.E., Kholmatova K.K., Gorbatova M.A., Grzhibovsky A.M. Application of survival analysis in healthcare using the SPSS statistical software package // Science and Healthcare. – 2017. – #5. – P.5-28. (*In Russian*).

[64] Shim J.Y., Kim M.H., Kim H.D., Ahn J.Y., Yun Y.S., Song J.Y. Protective action of the immunomodulator ginsan against carbon tetrachloride-induced liver injury via control of oxidative stress and the inflammatoryresponse // Toxicology and Applied Pharmacology. – 2010. – V.242(3). – P.318-325.

[65] Shiryaeva A.P., Baidyuk E.V., Arkadieva A.V., Okovitiy S.V., Morozov V.I., Sakut G.A. State of liver mitochondrial respiratory chain in rats with experimental toxic hepatitis // Cell and Tissue Biology. – 2007. – V.1(2). – P.169-177.

[66] Shorina E.D., Koshkina D.A., Grebnev D.Yu., Modeling of Liver Fibrosis in Mice // Proceedings of the VI International (76^{th} All-Russian) Scientific and Practical Conference "Topical Issues of Modern Medical Science and Health Care". – 2021. – P.1515-1520.

[67] Singh D., Khan M.A., Siddique H.R. Unveiling the therapeutic promise of natural products in alleviating drug-induced liver injury: Present advancements and future prospects // Phytotherapy Research. – 2023. – V.39(1). – P.22-41.

[68] Slater T.F. Activation of carbon tetrachloride: chemical principles and biological significance (In: "Free radicals, lipid peroxidation and cancer", McBrien D.C.H., Slater T.F. (Eds.) // Academic Press, London, 1981. – P.243-270.

[69] Slinin A.S., Bydanov O.I., Karachunsky A.I. Analysis of survival and the probability of occurrence of individual events in patients with acute leukemia // Issues of hematology/ oncology and immunopathology in pediatrics. – 2016. – #15(3). – P.34-39. (*In Russian*).

[70] Soguyko Yu.R., Krivko Yu.Ya., Krikun E.N. Ultrastructural features of the liver of rats in norm and in experimental diabetes mellitus at late stages of the course // Scientific news (Series Medicine. Pharmacy). – 2013. – #4(147). – Issue. 21. – P.147-150. (*In Russian*).

[71] Tabet E., Genet V., Tiaho F., Lucas-Clerc C., Gelu-Simeon M., Piquet-Pellorce C., Samson M. Chlordecone potentiates hepatic fibrosis in chronic liver injury induced by carbon tetrachloride in mice // Toxicol. Lett. – 2016. – V.25(255). – P.1-10.

[72] Timonin A.N., Nikitin N.S., Apryatin S.A., Trebukh M.D. Analysis of survival of ICR-1 mice in a biomodeling of starvation using carbon tetrachloride in an in vivo experiment // Issues of nutrition. – 2018. – #.87(5). – P.267-268. (*In Russian*).

[73] Titova A.A., Bilyalov A.I., Kiyasov A.P., Titova M.A. Laboratory animals for scientific research // Kazan (Kazan University), 2021. – P.71. (*In Russian*).

[74] Udut V.V., Vengerovsky A.I., Korshunov D.A., Karkishchenko N.N. The Effect of Phospholipid Hepatoprotectors on Bioenergetics and Lipid Peroxidation in the Liver in Experimental Pathology Induced by Paracetamol // Biomedicine. – 2012. – №1. – P.120-127.

[75] Wang B., Cui S., Mao B., Zhang Q., Tian F., Zhao J., Tang X., Chen W. Cyanidin Alleviated CCl_4-induced acute liver

injury by regulating the Nrf_2 and NF-κB signaling pathways // Antioxidants. – 2022. – V.11(2383). – P.1-14.

[76] Xue Y., Deng Q., Zhang Q., Ma Z., Chen B., Yu X., Peng H., Yao S., Liu J., Ye Y., Pan G. Gigantol ameliorates CCl_4-induced liver injury via preventing activation of JNK/$cPLA_2$/12-LOX inflammatory pathway // Scientific Reports. – 2020. – V.10(22265). – P.1-13.

[77] Yakovenko E.P., Yakovenko A.V., Ivanov A.N. et al. Drug-Induced Liver Damage. Diagnostics and Treatment // Attending Physician. – 2011. – #2. – P.16-20. (*In Russian*).

[78] Yu C., Wang F., Jin C., Wu X., Chan W.-K., McKeehan W.L. Increased carbon tetrachloride-induced liver injury and fibrosis in FGFR4-deficient mice // American Journal of Pathology. – 2002. – V.161(6). – P.1-8.

[79] Zapadnyuk I.P., Zapadnyuk V.I., Zakharia E.A., Zapadnyuk B.V. Laboratory Animals. Breeding, maintenance, experimental use (3rd edition, revised and enlarged) // Kyiv. – Publishing house "Vishcha shkola", 1983. – 380 p. (*In Russian*).

[80] Zhang L., Liu C., Yin L., Huang C., Fan S. Mangiferin relieves CCl_4-induced liver fibrosis in mice // Scientific Reports. – 2023. – V.13(4172). – P.1-9.

[81] Zhurakulov Sh.N. Tetrahydroisoquinoline, quinazolone alkaloid, its synthesizing and modification in biology // Thesis of dissertations by doctors (DSc). – Tashkent, 2023. – P.5-74. (*In Uzbek*).

[82] Zhurakulov Sh.N., Babkin V.A., Chernyak E.I., Morozov S.V., Grigorev I.A., Levkovich M.G., Vinogradova V.I. Aminomethylation of 1-aryl-6,7-dimetoxy-1,2,3,4-tetrahydroisoquinolines by dihydroquercetin // Chemistry of Natural Compounds. – 2015. – V.51(1). – P.57-61.

[83] Zhurakulov Sh.N., Vinogradova V.I., Levkovich M.G. Synthesis of 1-aryltetrahydroisoquinoline alkaloids and their analogs // Chemistry of Natural Compounds. – 2013. – V.49(1). – P.70-74.

Copyright © 2026 The Author(s). Published by Scientific & Academic Publishing
This work is licensed under the Creative Commons Attribution International License (CC BY). http://creativecommons.org/licenses/by/4.0/