ВЛИЯНИЕ НИЗКОМОЛЕКУЛЯРНОГО ХИТОЗАНА НА ТЕЧЕНИЕ ЭКСПЕРИМЕНТАЛЬНОГО ОСТРОГО ТОКСИЧЕСКОГО ГЕПАТИТА

Галиева Зульфия Ибрахимовна
Иноятова Фируза Хидоятовна – д.б.н., профессор
Таджиева Хосият Султановна – к.х.н., доцент
Ташкентская Медицинская Академия (Ташкент, Узбекистан)
Милушева Ракия Юнусовна – к.х.н.
Рахманова Вазира - Ph.D.
Институт Химии и Физики Полимеров АН Руз., (Ташкент, Узбекистан)

У 30 крыс с острым токсическим гепатитом, вызванным введением CCl_4 в оливковом масле в дозе 2,5 мг/кг массы тела в течение 4 дней, были изучены биохимические показатели повреждения печени. Для воспроизведения острого токсического гепатита 30 крысам вводили CCI_4 4 раза в дозе 2,5 мл/кг массы тела подкожно в течение 4 дней. Смертности не наблюдалось. Фармакотерапию острого токсического поражения печени проводили через 24 часа после окончательного введения токсиканта. Животные были разделены на следующие группы: контрольная группа (10 крыс) с ОТГ + плацебо (H_2O); группа сравнения (10 крыс) получала ОТГ + препарат сравнения Карсил в дозе 100 мг/кг; основная группа (10 крыс) получала ОТГ + НМХ в дозе 25 мг/кг. Препараты вводили в виде суспензии внутрижелудочно через специальную пробирку в течение 12 дней. В контрольной группе установлено развитие синдромов цитолиза, холестаза, мезенхимального воспаления и печеночно-клеточной недостаточности, снижение активности каталазы и увеличение содержания MDA, FASL и NF-кВ, что совпало с морфологическими признаками деструкции гепатоцитов. Низкомолекулярный хитозан, полученный из куколок Bombyx mori, в определенной степени восстанавливал гистиоструктуру печени крыс с АТГ, снижал процессы перекисного окисления липидов и апоптоза, цитолиза, холестаза и гепатоцеллюлярной недостаточности. По гепатопротекторному действию он не уступал классическому гепатопротектору Карсилу.

***Ключевые слова**: печень, четыреххлористый углерод, низкомолекулярный хитозан, карсил.*

КАМ МОЛЕКУЛЯР ОҒИРЛИКДАГИ ХИТОЗАННИНГ ЭКСПЕРИМЕНТАЛ ЎТКИР ТОКСИК ГЕПАТИТ ЖАРАЁНИГА ТАЪСИРИ

Галиева Зулфия Ибрагимовна
Иноятова Фируза Ҳидоятова – б.ф.д., профессор
Тожиева Хосият Султановна – к.ф.н., доцент
Тошкент Тиббиёт Академияси (Тошкент, Ўзбекистон)
Милушева Ракия Юнусовна – к.ф.н.
Рахманова Вазира - Ph.D.
Ўзбекистон Фанлар Академияси Полимерлар Кимёси ва Физикаси Институти (Тошкент, Ўзбекистон)

30 кун давомида зайтун мойида CCl_4ни 2,5 мг/кг тана вазнига дозада юбориш натижасида келиб чиққан ўткир токсик гепатит билан касалланган 4 каламушда жигар шикастланишининг биокимёвий кўрсаткичлари ўрганилди. Ўткир токсик гепатитни кўпайтириш учун 30 каламушга CCl_4 4 марта 2,5 мл / кг дозада 4 кун давомида тери остига юборилди. Ўлим кузатилмади. Жигарнинг ўткир токсик шикастланишининг фармакотерапияси токсикант охирги киритилгандан 24 соат ўтгач амалга оширилди. Ҳайвонлар қуйидаги гуруҳларга бўлинган: ОТГ + плацебо (H_2O) билан назорат гуруҳи (2 каламуш); таққослаш гуруҳи (10 каламуш) ОТГ + карсил таққослаш препаратини 100 мг/ кг дозада олди; асосий гуруҳ (10 каламуш) ОТГ + НМҲ ни 25 мг / кг дозада олди. Препаратлар 12 кун давомида махсус пробирка орқали интрагастрик равишда суспензия сифатида юборилган. Назорат гуруҳида ситолиз, холестаз, мезенхимал яллиғланиш ва жигар ҳужайралари етишмовчилиги синдромларининг ривожланиши, каталаза фаоллигининг пасайиши ва мда, ФАСЛ ва НФ-кб нинг кўпайиши аниқланди, бу гепатоцитлар йўқ қилинишининг морфологик белгиларига тўғри келди. Bombyx mori қўғирчоқларидан олинган паст молекуляр оғирликдаги хитосан атг билан каламушлар жигарининг гистиоструктурасини маълум даражада тиклади, липид пероксидацияси ва апоптоз, цитолиз, холестаз ва гепатоцеллуляр етишмовчилик жараёнларини камайтирди. Гепатопротектив таъсир жиҳатидан у классик гепатопротектор Карсилдан кам эмас эди.

***Калит сўзлар**: жигар, углерод тетрахлорид, паст молекуляр оғирликдаги хитозан, карсил.*

Introduction. Chitosan belongs to the group of biocompatible and biodegradable polymers, absolutely safe, non-toxic. This is a cationic aminopolysaccharide of natural origin, a copolymer of glucosamine and N-acetylglucosamine, which is obtained by partial deacetylation of chitin (β-(1–4)-Poly-N-acetyl-d-glucosamine) [15, Motya Azman]. Based on molecular weight, chitosan can be divided into chitosan with a high molecular weight of 190 to 375 kDa, with a deacetylation degree (DD) of more than 75%, and chitosan with a low molecular weight of 20 to 190 kDa, with a deacetylation degree of less than 75%. A high level of deacetylation indicates a low rate of dehydration, respectively, a low degree of deacetylation indicates a high level of dehydration [3, Dash M.; 5, Gauche A.]. With increasing molecular weight, the activity of chitosan increases. But despite this, chitosan with low and medium molecular weight exhibits higher antimicrobial properties [15, Mohammadi]. However, their hepatoprotective properties have not been studied.

Purpose of the research. To assess the hepatoprotective activity of low molecular weight chitosan obtained from pupae in a model of acute toxic liver damage.

Materials and Methods. Experimental studies were carried out following the requirements of the Declaration of Helsinki for the humane treatment of animals (Strasbourg, 1985) and the "Regulations on the use of laboratory animals in scientific research" atelier work and pedagogical process of Tashkent Medical Academy (TMA) and methods for implementing the requirements of biomedical ethics." To solve these problems, experiments were carried out on 40 mature male rats with an initial weight of 160-180 g, kept on a standard diet in the laboratory of pharmacology and toxicology of the Center for Biomedical Technologies (CBT) of the TMA. For the experiment, outbred white male rats of the same litter, the same age and body weight were used. They were kept in quarantine for a week and only after that were included in the experiments. The intact group consisted of 10 rats of the same litter. To reproduce acute toxic liver damage, 30 rats were administered CCI4 4 times at a dose of 2.5 ml/kg body weight subcutaneously for 4 days. No mortality was observed. Pharmacotherapy of acute toxic liver damage was carried out 24 hours after the final administration of the toxicant. The animals were divided into the following groups: control group (10 rats) with ATH+ placebo (H_2O); comparison group (10 rats) with ATH + comparison drug Carsil at a dose of 100 mg/kg; main group (10 rats) with ATH + LMWC at a dose of 25 mg/kg. The drugs were administered as a suspension intragastrically through a special tube for 12 days.

To assess the detoxifying function of the liver, an etaminal test was performed 24 hours after the final administration of drugs. The principle of the method is based on the fact that etaminal itself exhibits a hypnotic effect; after its metabolism in the liver by the cytochrome P450-dependent monooxygenase system, it loses its pharmacological properties. For this purpose, the animals were subcutaneously injected with etaminal sodium at a dose of 40 mg/kg and the time of falling asleep and waking up, the absence of the digestion reflex was recorded, and their difference (min) was calculated, which reflects the state of the detoxifying function of the liver. 24 hours after the test, the animals were decapitated under light ether anesthesia, and blood was collected for biochemical studies and liver for morphology. In the blood serum, the activity of alanine (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), the content of total protein, albumin, glucose, cholesterol, bilirubin, urea, creatinine, uric acid was determined on a biochemical analyzer MINDRAY BA-88A (China) using special test kits from the company in addition, catalase activity [9, Koralyuk M.A.], the content of malondialdehyde (MDA) [1, Andreeva L.I.], as well as the content and by the enzyme immunoassay method on an enzyme immunoassay analyzer ELISA (country) using test kits from the company. For histological studies, liver pieces were fixed in a mixture of 10% formalin, ethyl alcohol, and acetic acid, and embedded in paraffin. Sections 4–5 μm thick were stained with hematoxylin and eosin to assess the general structure. The preparations were visualized in Polyvar (Reichert-JUNG, Austria) and Leica DMRE (Leica Microsystems Wetzlar GmbH, Germany) microscopes with a digital video surveillance system and "Videotest-4" image analysis program. The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2012 software package, including the use of built-in statistical processing functions.

Results and Discussion. The studies showed that in the control group of rats, the duration of hexenal sleep was 180.33±5.97 minutes, while the value of this indicator in intact rats was 74.66±4.21, which exceeded the normative values by 2.42 times ($P<0.001$). In the comparison group (ATH+carsil), the duration of hexenal sleep was 95.17±4.47 minutes, which is 1.89 times ($P<0.01$) shorter than the indicators of the control group of animals, but 1.27 times ($P<0 .05$) longer than the values of intact rats. The use of LMWC at a dose of 25 mg/kg for 12 days contributed to a shortening of sleep duration, amounting to 114.17±5.47 minutes. This was 1.58 times ($P<0.01$) shorter than the values of the control group, but still significantly exceeded the values of

the comparison group and intact rats by 1.2 (P>0.05) and 1.53 times (P<0.05), respectively.

Thus, we can say that LMWC increases the pharmacometabolizing function of the liver of rats with ATH and its effect is comparable to the well-known hepatoprotector carsil.

Analysis of biochemical parameters showed that in rats of the control group with ATH in the blood serum, there was a tendency towards a decrease in the content of total protein (see Table 1). NMH slightly increased its level, while carsil did not have such an effect. In ATH rats, the albumin content decreased statistically significantly by 1.39 times (P<0.05) relative to the values of intact rats. When pharmacotherapy of ATH with the reference drug carsil, we observed only a tendency towards an increase in the level of albumin in the blood serum relative to the values of the untreated group, however, they remained statistically significantly lower by 1.27 times (P<0.05) than the indicators of intact rats. The direction of changes in the content of albumin in the blood serum was the same in the group of rats receiving LMWC at a dose of 25 mg/g. This indicator remained lower by 1.27 (P<0.05) times relative to the values of the intact group of rats.

Table 1.

Effect of hepatoprotectors on protein metabolism in the blood serum of rats with acute carbon tetrachloride hepatitis, M±m, n=10

Groups	Indicators of the protein spectrum of blood serum, g/l			
	Total protein	albumins	globulins	A/G coefficient
intact	68,48±2,92	40,63±0,47	27,84±2,90	1,55±0,12
ATH+H_2O	60,49±1,83	29,28±2,23[a]	31,21±3,48	1,08±0,19 [a]
ATH+carsil	60,33±2,74	32,01±2,16 [a]	28,31±1,95	1,18±0,13 [a]
ATH+LMWC	65,66±2,85	32,03±2,16 [a]	33,64±3,00	1,03±0,15 [a]

Note: *a – reliability of differences relative to the indicators of the intact group; b - reliability of differences relative to the control group; c – reliability of differences relative to the indicators of the comparison group.*

The content of globulins in the blood serum of rats with ATH tended to increase, indicating the presence of pronounced inflammatory processes in the liver parenchyma of experimental animals. The comparison drug carsil brought the studied indicator closer to the values of intact rats, while when using LMWC its values remained at the level of the control group of animals. The developing dysproteinemia contributed to a decrease in the albumin/globulin ratio. In intact rats, this indicator was 1.55±0.12. At the same time, this ratio in rats with ATH decreased by 1.43 times (P<0.05), indicating a fairly pronounced decrease in the content of the synthetic function of hepatocytes against the background of activation of stellate cells and the presence of inflammatory processes in the liver of experimental animals. During experimental pharmacotherapy with the reference drug carsil, this indicator only tended to increase and remained below 1.32 times (P<0.05) compared to the indicators of the intact group of rats. This indicates the persistence of structural changes in the liver parenchyma of rats in this group and was mainly associated with low levels of albumin. The use of LMWC did not have a noticeable effect on the A/G coefficient. This indicator remained within the values of the control group of animals and was significantly lower by 1.51 times (P<0.05) than the indicators of the intact group of animals. This was mainly due to the persistence of high globulin values, indicating a weak effect of this drug in the correction of ATH.

Next, we studied the effect of chitosan derivatives on the biochemical parameters of blood serum (see Table 2). Studies have shown that ATH rats develop severe hyperbilirubinemia, manifested by an increase in the level of total bilirubin in the blood serum by 3.66 times (P<0.001) relative to the values of the intact group of rats. Considering that the causes of hyperbilirubinemia can be various etiological factors, it can be assumed that in this case it is associated with damage to the liver parenchyma with a violation of its bilirubin-secreting function, specifically with a violation of the activity of the enzyme unit that ensures the biosynthesis of bilirubin glucuronides and the hepatic secretion of conjugated bilirubin into bile. Pharmacotherapy with ATH carsil led to a significant decrease in the content of total bilirubin in the blood serum of rats by 3 times (P<0.001) and approaching the values of intact rats. This was due to the presence of silymarin in the composition of carsil. Its mechanism of action is associated with the stabilization of hepatocyte membranes, acceleration of repair processes, neutralization of free radicals, activation of systemic processes, and slowing down the penetration of certain hepatotoxins into liver cells [2, Antonova T.V.; 13, Matveeva A.V.]. When using LMWC, the content of total bilirubin in the blood serum of rats with ATH decreased statistically significantly by 2.31 times (P<0.001) relative to the values in the control group. However, this indicator remained higher than the values of the intact group of rats by 1.58 times (P<0.01).

Table 2.

Influence of hepatoprotectors on biochemical parameters of blood serum of rats with acute carbon tetrachloride hepatitis, M±m, n=10

Groups	Biochemical parameters of blood serum			
	Total bilirubin, μmol/l	Glucose, mmol/l	Triglycerides, mmol/l	Total cholesterol, mmol/l
intact	9,25±0,52	5,04±0,27	0,54±0,07	2,60±0,23
ATH+H_2O	33,81±2,41 [a]	5,54±0,29	1,00±0,06 [a]	5,40±0,40 [a]
ATH+carsil	11,31±1,09 [b]	5,18±0,35	0,52±0,04 [b]	2,81±0,26 [b]
ATH+LMWC	14,65±1,54 [a,b]	3,97±0,29 [a,b]	0,64±0,06 [b]	3,61±0,17 [a,b]

Note: *a – reliability of differences relative to the indicators of the intact group; b - reliability of differences relative to the control group; c – reliability of differences relative to the indicators of the comparison group.*

The glucose content in the blood serum of rats with ATH tended to increase relative to the values of intact rats. Pharmacotherapy with ATH carsil did not have a noticeable effect on glucose levels. At the same time, when using LMWC, we observed its decrease by 1.39 times (P<0.05) relative to the values of the control group of rats and by 1.27 times (P<0.05) relative to the values of intact rats. In our opinion, this is due to its hypoglycemic effect [8, Konstantin S. M.]. The mechanism of its hypoglycemic effect is related to the regulatory role of the main pathways of glucose into the liver and muscles. The effectiveness of chitosan and its derivatives depends on their size and molecular weight. In the research of Naveed M. et al. (2019), Yu S.Y. et al. (2017) showed the expression of the glucose transporter 4 (GLUT4) gene in diabetic rats with increased glycogen production in the liver [16, Naveed M.; 17, Yu S.Y.].

The content of triglycerides in the blood serum of rats with ATH increased statistically significantly by 1.85 times (P<0.01) relative to the values of intact rats. This is due to a decrease in VLDL in the liver, which transfers endogenous TAG to the blood and organs, and tissues. It should be said that all study drugs reduced high levels of TAG. Thus, with pharmacotherapy with carsil, this decrease was 1.92 times (P<0.001), with the use of LMWC - 1.56 times (P<0.01) relative to the indicators of the control group of animals. At the same time, LMWC was somewhat inferior to carsil and approached the values of intact rats.

In the blood serum of rats with ATH, the level of total cholesterol sharply increased, exceeding the indicators of the intact group of rats by 2.08 times (P<0.001). Indeed, according to literature data, the cholesterol content in blood serum increases in liver diseases, due to a decrease in the synthetic function of hepatocytes, in the endoplasmic reticulum of which primary bile acids are synthesized from cholesterol. On the other hand, with acute and chronic liver damage, due to compression of the bile ducts by edema of hepatocytes and inflammatory processes, intrahepatic cholestasis increases, which also leads to the development of hypercholesterolemia. Pharmacotherapy with ATH carsil for 12 days contributed to a significant decrease in total cholesterol in the blood serum of experimental animals by 1.92 times (P <0.001) and approaching the values of intact rats. This was due to the choleretic effect of silymarin, which is part of carsil. The effect of chitosan was manifested to a lesser extent. Thus, the content of total cholesterol in the blood serum of rats with ATH treated with LMWC statistically significantly decreased by 1.5 times (P < 0.05). Despite this decrease, the level of total cholesterol in the blood serum of this group of animals exceeded the indicators of intact animals by 1.39 times (P<0.05).

Thus, various chitosan derivatives reduced the severity of hyperbilirubinemia, hypertriglyceridemia, and hypercholesterolemia characteristic of rats with toxic liver damage. Studies by Inoyatova F.Kh., Milusheva R.Yu., Kutlikova G. (2016-2018) showed the effectiveness of chitosan and, especially its sulfated form, in reducing the risk of atherogenesis, which was confirmed by low values of total cholesterol and cholesterol in very low and low-density lipoproteins in rats with hypercholesterolemia [12, Kutlikova G.M.]. According to the authors, they suppressed high levels of endothelin-1, homocysteine, and CRP, and increased eNOS activity and nitric oxide content, which coincided with its pronounced lipid-lowering properties.

Analysis of nitrogen metabolism parameters in the blood serum of rats with ATH showed the development of hyperazotemia, manifested by an increase in the amount of urea, creatinine, and uric acid (see Table 3). Thus, the urea content in the blood serum statistically significantly increased by 2.4 times (P<0.001) relative to the values of the intact group of rats. This, in our opinion, is associated with the production of azotemia, due to increased cell breakdown due to the development of inflammatory-necrotic processes. This is confirmed by the tendency to increase the level of creatinine in the blood serum of this group of animals, as well as a significant increase of 2.96 times (P<0.001) in the content of uric acid. As you know, urea is the end product of protein metabolism in the body. It is removed from the body through glomerular filtration, 40-50% of it is reabsorbed by the tubular epithelium of the kidneys and is actively secreted by tubular cells. Therefore, in pathology, shifts in the concentration of urea in the

blood depend on the ratio of the processes of its formation and excretion. At the same time, uric acid is a product of the metabolism of purine bases. In this case, the developed hyperuricemia, in our opinion, is secondary, and associated with damage to the liver and biliary tract. As can be seen from the presented material, the hyperazotemia observed in rats with OTH is production hyperazotemia.

Pharmacotherapy with carsil in rats with ATH contributed to a decrease in the content of urea, creatinine, and uric acid by 1.83 (P<0.01); 1.13 (P>0.05) and 1.79 times (P<0.01), respectively, relative to the values of the control group. Moreover, if the level of creatinine approached the values of the intact group of rats, then the content of urea and uric acid was significantly 1.31 (P<0.05) and 1.65 times (P<0.01) higher than the values of intact rats. This, in all likelihood, was associated with a certain degree of reduction in inflammatory-necrotic processes in the liver.

Table 3.

Effect of hepatoprotectors on residual nitrogen levels in the blood serum of rats with acute carbon tetrachloride hepatitis, M±m, n=10

Groups	Urea, mmol/l	Creatinine, μmol/l	Uric acid, mmol/l
intact	4,60±0,38	66,79±0,93	0,26±0,01
ATH+H_2O	11,05±0,55 [a]	75,03±3,56	0,77±0,03 [a]
ATH+carsil	6,04±0,48 [a,b]	66,60±2,26	0,43±0,02 [a,b]
ATH+LMWC	7,95±0,65 [a,b]	71,69±3,24	0,47±0,03 [a,b]

Note: *a – reliability of differences relative to the indicators of the intact group; b - reliability of differences relative to the control group; c – reliability of differences relative to the indicators of the comparison group.*

Pharmacotherapy with LMWC in rats with ATH for 12 days led to a significant decrease in urea and uric acid levels by 1.39 (P<0.05) and 1.63 times (P<0.01), without having a significant effect on creatinine levels. Despite such positive changes in the level of urea and uric acid in the blood serum of rats remained significantly 1.73 (P<0.01) and 1.81 times (P<0.01), respectively.

The next stage of our research was to assess the effect of drugs on the activity of liver enzymes (see Table 4). It was found that in rats with ATH, ALT activity increased statistically significantly by 1.83 times (P<0.01) relative to the values of the intact group of rats. An increase in the activity of this enzyme indicates cytolysis of hepatocytes since it reaches its highest concentrations in the liver. The degree of increase in aminotransferase activity indicates the severity of the cytolytic syndrome but does not directly indicate the depth of the violation of the organ function itself. AST activity in the blood serum due to acute toxic hepatitis statistically significantly increased by 2.73 times (P<0.001), which is typical for carbon tetrachloride hepatitis, which mainly damages the mitochondria of hepatocytes. Alkaline phosphatase activity increased 3.31 times (P<0.001), indicating destruction of hepatocytes (hepatic cell mechanism) or disruption of bile transport (cholestatic mechanism). The hepatic cell mechanism of increasing alkaline phosphatase activity plays a leading role in toxic and drug-induced liver damage. In some cases, alkaline phosphatase activity increases due to the simultaneous action of both mechanisms of damage. In our opinion, with toxic hepatitis, is both due to hepatic and cholestatic mechanisms. GGTP activity in rats with ATH statistically significantly increased by 3 times (P<0.001) relative to the values in intact rats. This enzyme is mainly localized in the membranes of microsomes and the cytoplasmic membrane of cells, therefore, an increase in GGTP activity in the blood serum is of great diagnostic importance for diseases of the liver and hepatobiliary tract. As can be seen from the data presented, the ATH model we used is adequate and is characterized by all developing syndromes of liver damage.

Experimental pharmacotherapy with carsil in rats with ATH for 12 days led to a decrease in the activity of the studied enzymes in the blood serum of rats. Thus, the activity of ALT, AST, ALP, and GGTP decreased by 1.62 (P<0.05); 1.2; 1.43 (P<0.05), and 1.84 times (P<0.01), respectively. If ALT activity somewhat approached the values of intact rats, AST, ALP, and GGTP remained above the values of the intact group of rats at 2.28 (P<0.001); 2.32 (P<0.001) and 1.64 times (P<0.01) exceeded them (see Table 4). This indicates the persistence of dystrophic changes in hepatocytes and cholestasis in experimental animals.

Table 4.

Effect of hepatoprotectors on the activity of serum enzymes in rats with acute carbon tetrachloride hepatitis, M±m, n=10

Groups	Enzyme activity, IU/l			
	ALT	AST	alkaline phosphate	GGTP
intact	58,26±2,81	51,10±5,06	289,25±23,93	38,38±2,19
ATH+H_2O	106,68±6,36 [a]	139,44±3,02 [a]	957,5±72,6 [a]	115,50±9,26 [a]

ATH+carsil	65,70±5,64 b	116,55±6,81 a,b	671,2±67,2 a,b	62,88±3,99 a,b
ATH+LMWC	55,04±4,30 b	110,13±5,72 a,b	635,9±80,2 a,b	68,38±3,98 a,b

Note: *a – reliability of differences relative to the indicators of the intact group; b - reliability of differences relative to the control group; c – reliability of differences relative to the indicators of the comparison group.*

Experimental pharmacotherapy with LMWC for 12 days in rats with ATH led to a decrease in the activity of ALT, AST, ALP, and GGTP by 1.94 (P<0.001); 1.27 (P<0.05); 1.51 (P<0.05) and 1.69 times (P<0.01), respectively, relative to the values of the untreated group of rats. If ALT activity approached the values of intact rats, the activities of AST, ALP, and GGTP remained above the values of the intact group of rats by 2.15 (P<0.001); 2.2 (P<0.001) and 1.78 (P<0.01) times higher than them. These values did not differ significantly from the indicators of the comparison group and indicated the persistence of inflammatory and infiltrative processes in the liver of experimental animals.

Thus, in rats with ATH, hypoalbuminemia develops against the background of increased globulins, productive hyperazotemia, hypertriglyceridemia, hypercholesterolemia, and increased liver enzymes. The data obtained indicate the development of syndromes of cytolysis, cholestasis, mesenchymal inflammation, hepatic cellular failure, and a decrease in the detoxifying function of the liver. This is due to the toxic effect of trichloromethane radicals. Experimental pharmacotherapy with carsil and LMWC leads to a certain extent to an increase in albumin content, a decrease in the level of globulins, and residual nitrogen indicators. Along with this, the drugs reduced the severity of triglyceridemia, hypercholesterolemia, and hyperfermentemia.

To clarify some aspects of the mechanism of the hepatoprotective action of LMWC in blood serum, we studied the indicators of lipid peroxidation. Analysis of the MDA content in the blood serum of rats with acute toxic hepatitis showed a significant increase of 172.9% (P<0.001) relative to the values of intact rats (see Table 5). It is known that the administration of CCl4 to rabbits leads to early destruction of cytochrome P-450 of liver microsomes, inhibition of the enzyme glucose-6-phosphatase, ultrastructurally detectable intense necrosis and fatty degeneration of the liver. Metabolized in the liver, carbon tetrachloride forms active metabolites that covalently bind to lipids, inducing lipid peroxidation. On the other hand, the covalent binding of carbon tetrachloride metabolites to cellular elements is more important in liver damage than lipid peroxidation.

It is well known that the function of catalase is to protect the body from reactive oxygen radicals and hydrogen peroxide. Analysis of the activity of this enzyme showed its decrease by 52.1% (P<0.01), indicating that a decrease in the activity of antioxidant enzymes plays an important role in the development of hyperlipid peroxidation. Indeed, the ratio of catalase activity to MDA level in intact rats was 19.10±1.57, while in rats with ATH, it was 3.15±0.21, which is 5.67 (P<0.001) times lower than the values in intact rats. Consequently, under the influence of active carbon tetrachloride radicals, a decrease in the activity of antioxidant enzymes leads to the destruction of biomembranes and the development of cytolysis of hepatocytes.

Table 5.

Malondialdehyde content and catalase activity in the blood serum of experimental animals, M±m, n=10

Groups	MDA content, nmol/ml		Catalase activity, mkat/l	
	M±m	P_1/P_2	M±m	P_1/P_2
Intact	1,520±0,107		28,13±1,77	
ATH+H_2O	4,253±0,176	0,001	13,32±0,91	0,01
ATH+carsil	2,288±0,132	0,02 0,002	20,46±0,97	0,03 0,02
ATH+LMWC	2,675±0,153	0,02 0,01	17,98±1,56	0,02 0,05

Note: *P1 – reliability of differences between the indicators of the intact and experimental groups, P2 – reliability of differences between the indicators of the treated and untreated groups.*

Experimental pharmacotherapy with carsil in rats with ATH contributed to a decrease in MDA content by 44.2% (P<0.002) relative to the values of the control group. However, despite such positive changes, the level of MDA remained higher by 52.3% (P<0.02) compared to the indicators of the intact group of rats. At the same time, catalase activity increased statistically significantly by 51.1% (P<0.02) relative to the values of the control group but was still lower by 27.7% (P<0.03) than the values of the intact group of rats. The index of catalase activity/MDA level was 9.15±0.21, which is 2.69 (P<0.001) times higher than the values of the control group, but remained lower than the values of the intact group of rats by 2.11 times (P<0.001).

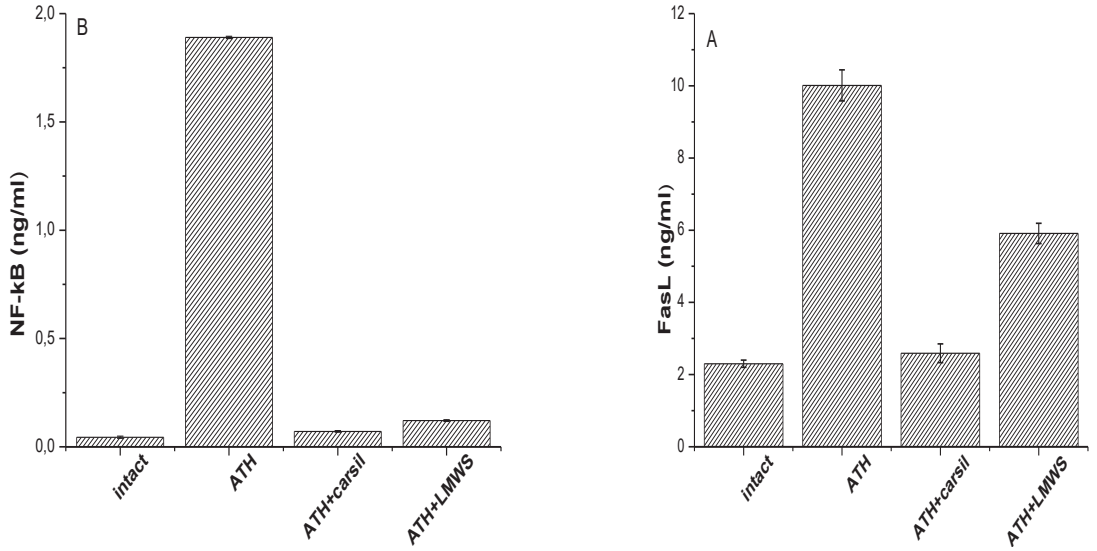
Pharmacotherapy with LMWC in rats with ATH contributed to a decrease in MDA content by 40.4% (P<0.01) relative to the values of the un-

treated group, but still exceeded the values of intact rats by 62.6% (P<0.02). Catalase activity increased by 29.9% (P<0.05) but remained below the values of the intact group of rats by 37.8% (P<0.02). The ratio of catalase activity/MDA content in this group was 6.44±0.59, which is 2.17 (P<0.001) times higher than in the control group. However, this indicator remained lower than the values of intact rats by 2.61 times (P<0.001). It should be said that the antioxidant effectiveness of LMWC was lower than the values of the comparison group.

Thus, in rats with ATH, hyperlipid peroxidation was established due to a decrease in catalase activity. The antioxidant effectiveness of LMWC was lower than that of the group of animals receiving carsil.

Fas ligand, known as the "death factor", binds to the Fas receptor and induces cell death. The Fas-FasL system initiates the destruction of autoreactive T cells and the development of hepatitis. The membrane-bound Fas ligand is converted into a soluble form by metalloproteinase. Taking into account the above, we determined the content of FasL in the blood serum of experimental animals using the enzyme immunoassay method. The studies showed that the FasL content in intact rats was 2.30±0.10 ng/ml (Fig. 1). In rats with ATH, this indicator statistically significantly increased by 4.35 (P<0.001) times and amounted to 10.01±0.43 ng/ml, indicating activation of the receptor-mediated apoptosis mechanism.

According to the literature, in viral hepatitis, the "external" pathway of apoptosis mainly predominates, due to the initiation of "death receptors" on the surface of hepatocytes and cholangiocytes, in which the Fas–FasL interaction plays a leading role [7, Karev V.E.; 10, Kremer A.E.]. It should be said that along with this, the "internal" path is also activated [11, Kurbanova N.N.]. Immunohistochemical studies have shown that on the surface of T-lymphocytes that form the inflammatory infiltrate in the liver in chronic hepatitis C (CHC), the expression of Fas ligand is increased, which can serve as an apoptotic signal for hepatocytes carrying the Fas receptor on their surface [4, Dmitrieva E.V.]. On the other hand, the accumulation of unrepaired DNA damage resulting from oxidative stress is one of the triggers of apoptosis [2, Antonova T.V.; 6, Hatano E.].



a b

Fig. 1. **The effect of carsil and low molecular weight chitosan on the content of FasL (ng/ml) (a) and NF-kB (ng/ml) (b) in the blood serum of rats with acute carbon tetrachloride hepatitis after 12 days of use.**

Experimental pharmacotherapy of ATH with the reference drug Carsil at a dose of 100 mg/kg contributed to a decrease in the FasL content in the blood serum of experimental animals by 4 (P<0.001) times relative to the values of the control group of rats and amounted to 2.59±0.26 ng/ml. At the same time, we observed their approach to the values of intact rats. When using LMWC, we also observed a decrease in FasL content in the blood serum of experimental animals. Thus, the FasL content in the blood serum of experimental animals decreased to 5.91±0.28 ng/ml, which is 1.69 (P<0.02) times lower than the control group. However, these indicators were still significantly higher by 2.57 (P<0.001) and 2.36 (P<0.001) times the values of intact rats and rats of the comparison group.

NF-kB is one of the main transcription factors responsible for adaptive cell responses. NF-kB is a family of cytoplasmic proteins that when stimulated, become free, moving into the nucleus, where they are active by binding to the promoter regions of more than 100 genes responsible for inductive homeosta-

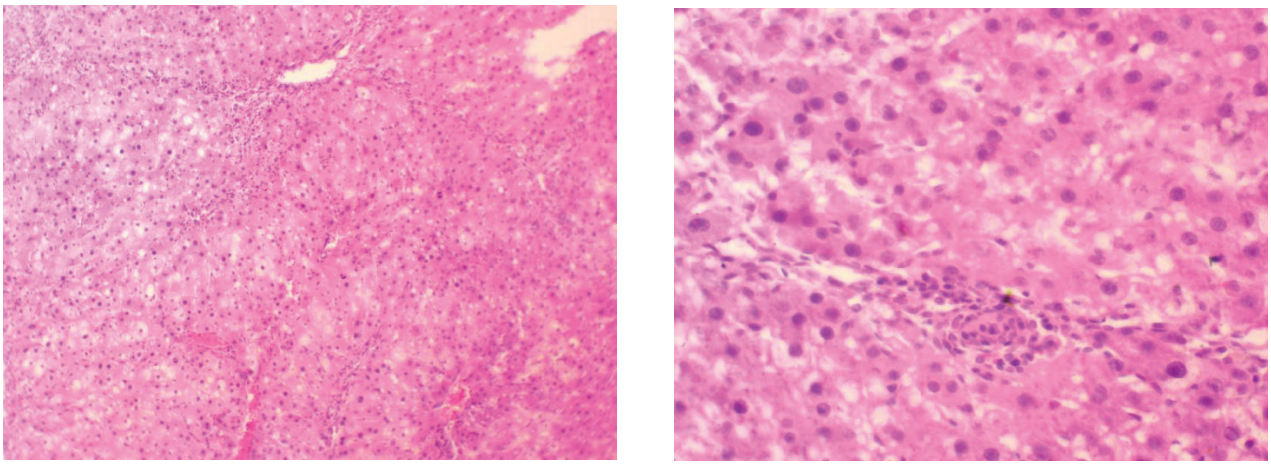
sis. NF-kB is present in the cytoplasm in an inactive form, being in a complex with inhibitory IkB proteins. When stimulated, NF-kB undergoes phosphorylation and ubiquitination, which, after additional phosphorylation, can migrate into the cell nucleus, to the site of its action. Transcriptional activity of NF-kB appears within minutes after stimulation. NF-kB has one of the central positions in the regulation of the inflammatory process. Considering the above, it was of interest to study its content in the blood serum of experimental animals.

The studies showed that the content of NF-kB in the blood serum of intact rats was 0.044±0.005 ng/ml (see Fig. 2). In ATH rats, the content of this factor increased sharply, amounting to 0.189±0.005 ng/ml, and exceeded the values of intact rats by 4.3 times (P<0.001). It should be said that activation of NF-kB increases the expression of adhesive molecules, and accelerates the synthesis of pro-inflammatory cytokines and inducible enzymes (NO-synthase, cyclooxygenase-2, collagenase, etc.). This protein mediates inflammatory and immune responses, responses to viral infections, cell division, and regulation of apoptosis. Activation of NF-kB usually delays apoptosis, prolonging the life of effector cells at the site of inflammation.

Experimental pharmacotherapy of ATH with the reference drug carsil at a dose of 100 mg/kg led to a decrease in the high level of NF-kB by 2.66 (P<0.001) times relative to the values of the control group, amounting to 0.071±0.004 ng/ml. Despite this decrease, the level of this factor in the blood serum of rats was significantly 1.61 (P<0.05) times the value of the intact group of rats. Pharmacotherapy with LMWC at a dose of 25 mg/kg also contributed to a decrease in the high values of experimental animals by 1.56 (P<0.05) times, amounting to 0.121±0.004 ng/ml. These values were 1.7 (P<0.01) times lower than the values of the comparison group and 2.75 (P<0.001) times lower than the values of the intact group of rats.

Therefore, HMX chitosan reduces the level of high levels of NF-κB in the serum of ATH rats.

In order to determine the effectiveness of hepatoprotectors included in the diet of experimental animals depending on the dose, we conducted a histological study of liver tissue. A morphological study of the liver of experimental animals after treatment with various drugs in the experimental and control groups revealed the following changes. Thus, in the control group of animals with ATH, which received a placebo for 12 days, the morphological picture was characterized by the presence of diffuse periportal mesenchymal cell infiltration, lysis, and fatty transformation of hepatocytes with nuclear displacement. In several lobes of the liver, dusty fatty degeneration of hepatocytes was detected under low magnification (Fig. 2).



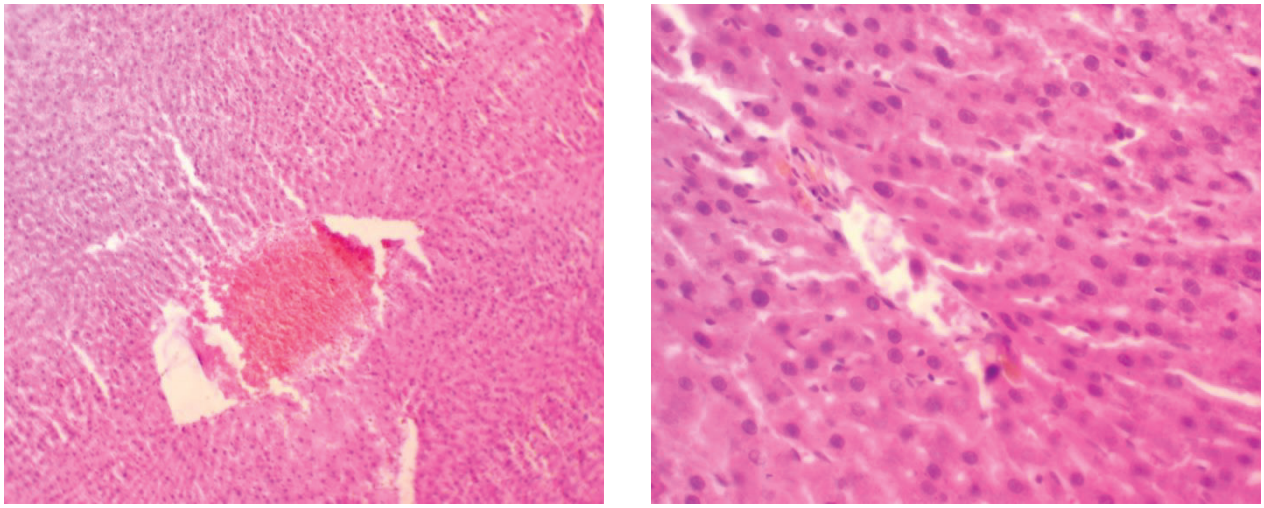
a **b**

Fig. 2. **Rat liver with ATH. Under low magnification of the lens, liver lobules with slit-like narrowing of the lumen of blood vessels, small-droplet fatty degeneration of hepatocytes (a). Zoom:10.0. Hematoxylin and eosin staining. Under high lens magnification, periportal cellular infiltration, lysis, and fatty transformation in hepatocytes are noted (b). Hematoxylin and eosin staining. Zoom: 40.0.**

Under low magnification of the lens, liver lobules with slit-like narrowing of the lumen of blood vessels, and small-droplet fatty degeneration of hepatocytes. In the liver lobules, the central vein is dilated, and the beam structure of hepatocytes is preserved. In the periportal tracts, there is a cellular reaction and isolated fatty inclusions. At high magnification, periportal cellular infiltration, lysis of individual hepatocytes, and fatty transformation in hepatocytes are observed. In rats with acute toxic liver damage, a decrease in the recovery response and increased signs of the proliferation of immature connective tissue with an abundance of cellular structures were observed. The results obtained indicate

the adequacy of our chosen model of acute toxic liver damage.

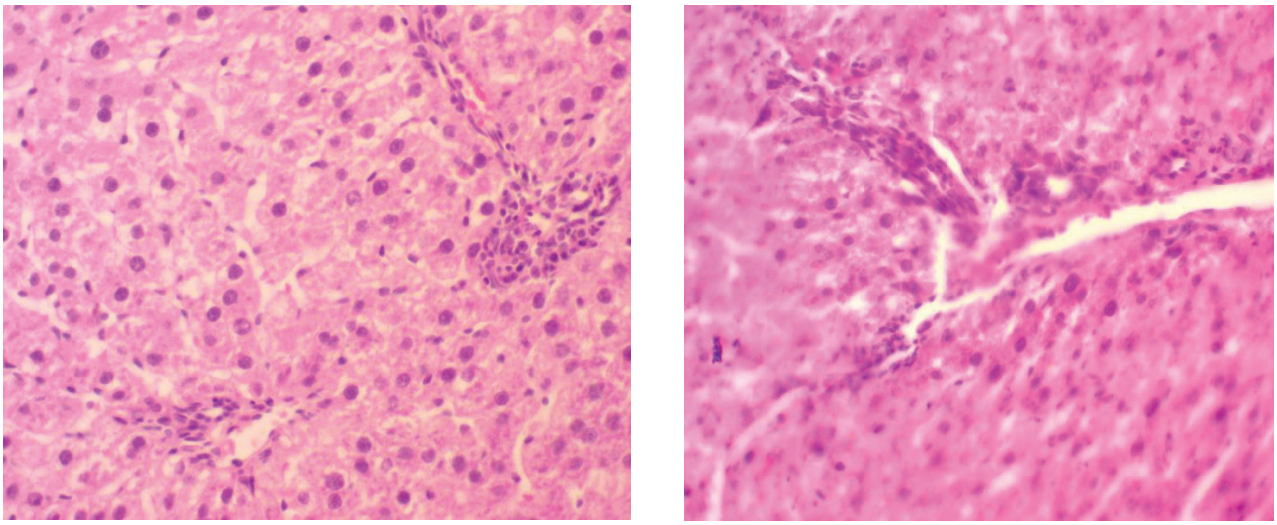
During experimental pharmacotherapy of ATH with the classical hepatoprotector carsil at a dose of 100 mg/kg for 12 days, signs of venous hyperemia, a sharp expansion of the lumen of the central vein, vascular congestion were noted, sinusoidal spaces and Kupffer cells were identified. Hepatocytes had a beam structure; in the middle sections of the lobule there were single hepatocytes with dusty fatty degeneration of a focal nature (Fig. 2a). In some places, the beam structure of hepatocytes had a parallel-oriented direction. Under high lens magnification, single mononuclear cells were identified in Disse space. Hepatocytes are round-polygonal in shape, the cytoplasm is pink-reddish in color, and their nuclei are hyperchromatically colored (Fig. 3).



a **b**

Fig. 3. **Liver of a rat with ATH treated with carsil. The central vein is sharply dilated, and full of blood, in the middle sections of the lobule there are single hepatocytes with dusty fatty degeneration of a focal nature (a). The beam structure of the liver is preserved, the nuclei in the hepatocytes are identified, and in the lobule the lumen of the central vein is expanded and contains red blood cells (b). Zoom: 40.0. Hematoxylin and eosin staining.**

In animals with ATH that underwent pharmacotherapy with LMWC under a high magnification lens, focal accumulation of cells of a histiogenic nature around the vessels and fatty inclusions in hepatocytes were detected. Signs of a mesenchymal-cellular reaction along the periportal tracts were noted, in the space of Disse there was an accumulation of lymphocytes, histiocytes, a slit-like lumen of blood vessels, as well as dystrophic changes in hepatocytes, Councilman's bodies, but the beam structure was preserved (Fig. 4).



a **b**

Fig. 4. **Liver of rats with ATH treated with LMWC. Focal accumulation of cells of histiogenic nature around the vessels; fatty inclusions in hepatocytes (a). Mesenchymal-cellular reactions along the periportal tracts, slit-like vessels, and hepatocytes are preserved (b). Hematoxylin and eosin staining. Zoom: 40.0**

Thus, morphological studies of the liver in rats with ATH showed the presence of a diffuse mesenchymal-cellular reaction, which is an irreversible process culminating in the synthesis of collagen fibers, sclerotic changes with subsequent pathological regeneration and the formation of false lobules, which is subsequently aggravated by portal hypertension. In the group of animals with OTH treated with the drug carsil, morphological studies indicated the preservation of signs of tissue hypoxia and stagnation. In the group of rats with ATH treated with LMWC, signs of hypoxia and activation of the mesenchymal cellular reaction were morphologically revealed, indicating the initial stage of sclerotic changes in the liver.

Based on the results obtained, the following conclusions can be drawn:

1.Low molecular weight chitosan obtained from pupae... to a certain extent restores the histiostructures of the liver of rats with ATH and is not inferior in morphological characteristics to the classical hepatoprotector carsil.

2.In the mechanism of the hepatoprotective action of LMWC, an important role is played by the activation of antioxidant defense enzymes, the slowdown of lipid peroxidation, and the reduction of FasL and NF-kB levels.

3.To a certain extent, restoration of the structure of biomembranes leads to activation of the pharmacometabolizing function of hepatocytes, a decrease in hyperenzymia and hyperbilirubinemia, and an increase in the protein-synthesizing function of hepatocytes.

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EFFECT OF LOW MOLECULAR WEIGHT CHITOSAN ON THE COURSE OF EXPERIMENTAL ACUTE LIVER DAMAGE

Galieva Zulfiya Ibrarkimovna
Inoyatova Firuza Khidoyatovna – D.B.Sc., professor
Tadjieva Khosiyat Sultanovna - D.Ch.Sc., associate professor
Tashkent Medical Academy (Tashkent, Uzbekistan)
Milusheva Rakiya Yunusovna – D.Ch.Sc.
Rakhmanova Vazira - Ph.D.
Institute of Polymer Chemistry and Physics Academy of Science of the Republic Uzbekistan (Tashkent, Uzbekistan)

Abstract. *In 30 rats with acute toxic liver damage caused by the administration of CCl4 in olive oil at a dose of 2.5 mg/kg body weight for 4 days, biochemical indicators of liver damage were studied. To reproduce acute toxic liver damage, 30 rats were administered CCI4 4 times at a dose of 2.5 ml/kg body weight subcutaneously for 4 days. No mortality was observed. Pharmacotherapy of acute toxic liver damage was carried out 24 hours after the final administration of the toxicant. The animals were divided into the following groups: control group (10 rats) with ATH + placebo (H_2O); comparison group (10 rats) with ATH + comparison drug Carsil at a dose of 100 mg/kg; main group (10 rats) with ATH + LMWC at a dose of 25 mg/kg. The drugs were administered as a suspension intragastrically through a special tube for 12 days. In the control group, the development of syndromes of cytolysis, cholestasis, mesenchymal inflammation, and hepatic cell failure, a decrease in catalase activity, and an increase in the content of MDA, FASL, and NF-kB was established, which coincided with the morphological signs of hepatocyte destruction. Low-molecular chitosan obtained from Bombyx mori pupae to a certain extent restored the histiostructure of the liver of rats with ATH, reduced the processes of lipid peroxidation and apoptosis, cytolysis, cholestasis and hepatocellular failure. In terms of hepatoprotective action, it was not inferior to the classical hepatoprotector Carsil.*

Keywords: *liver, carbon tetrachloride, low molecular weight chitosan, carsil.*