

Study of reparative processes and immune mechanisms in liver tissues and blood plasma of rats against the background of loading with plant extracts and induced oxidative stress

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Abstract. The aim of the study was to investigate reparative processes and immune mechanisms in rat liver tissues against the background of loading with aqueous extracts of blackberry and common sawfly and induced oxidative stress. 120 rats were used in the experiment. According to group affiliation animals received aqueous extracts of common and blackberry in a dose of 50 mg/100g of weight, 1.5 ml daily for 30 days, and animals of the control group received distilled water in the same mode. Starting from 30 days of the experiment, rats were injected with CCl₄ for 6 days. On the 37th day of the experiment, rats were killed according to ethical standards. The number of sinusoidal cells was counted in liver tissues. The content of cytokines in liver homogenates and blood plasma of rats was determined by enzyme-linked immunosorbent assay. Conclusion. Aqueous extracts of fir and blackberry modulate to different degrees the functional state of sinusoidal cells in early periods of toxic exposure to tetrachloromethane, which contributes to the early resolution of the inflammatory process. Exposure of aqueous extracts of fir and blackberry to sinusoidal cells changes the production of regulatory factors, which compensates the speed of recovery processes after toxic exposure.

1 Introduction

Diseases of the hepatobiliary system constitute a significant part of the overall disease burden. One of the most common causes leading to the development of these diseases is exposure to hepatotoxic substances that induce oxidative stress. Despite a large number of scientific publications, the role of immune mechanisms in the pathogenesis of oxidative stress development in diffuse toxic liver injury has not been definitively elucidated [1,2].

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The morphological changes that occur in the liver during toxic injury are based on hepatocyte cytolysis. This process initiates a progressive necrobiosis of liver cells. Excessive peroxidation of membrane structures caused by increased production of reactive oxygen species is a universal mechanism of damage and even cell death in any organ, including the liver [3,4].

Metabolic disorders resulting from tetrachloromethane exposure may alter the production of anti-inflammatory and anti-inflammatory cytokines by cells of the immune system. Liver sinusoidal cells, most of which are macrophages, play a particular role in this process. These cells have important functions in maintaining the inflammatory response and regulating the regeneration process [5,6].

Among the pathogenetic treatments of liver diseases, a group of hepatoprotectors, mainly of plant origin, with antioxidant properties, has traditionally been distinguished [7,8].

Therefore, the aim of our study was to investigate reparative processes and immune mechanisms in rat liver tissues against the background of loading with aqueous extracts of blackberry and tansy and induced oxidative stress [9-15].

2 Materials and Methods

In the experiment, 120 male rats were used, equally divided into 4 groups. Group 1: intact rats, no effects were applied to them; group 2: animals receiving distilled water and CCl₄ injections; group 3: rats receiving aqueous extract of blackberry and CCl₄ injections; and group 4 - rats receiving aqueous extract of tansy and CCl₄ injections. According to their group assignment, animals received aqueous extracts of tansy and blackberry at a dose of 50 mg/100 g of animal weight, 1.5 ml daily for 30 days, and animals in the control group received distilled water in the same regime. Starting from the 30th day of the experiment, rats were injected with tetrachloromethane oil solution at a dose of 2 g/kg of animal weight for 6 days in parallel with the administration of plant extracts. On day 37 of the experiment, the animals were blood sampled and then killed by decapitation under ether anaesthesia in accordance with ethical standards, followed by liver extraction. Liver tissue samples were immersed in 10% neutral formalin for 24 hours at room temperature. Histological preparations were prepared using a Leica EG 1160 automated processor. Liver sections were stained with haematoxylin and eosin. Microscopic examination was performed on a Leica DM 2500 microscope and images were analysed using Leica Application Suite (V4). The number of sinusoidal cells was counted per unit area in 20 fields of view at a microscope magnification of $\times 400$.

For analysis of cytokine content in blood plasma of experimental animals, peripheral blood was collected and centrifuged at 3000 rpm for 15 min in the cold. For analysis of cytokine content in liver tissue, homogenates were prepared by homogenising liver samples, resuspending with physiological solution (0.85% sodium chloride solution) and centrifuging at 5000 rpm in cold for 30 min [16]. Cytokine levels in rat liver homogenates and blood plasma were determined by enzyme-linked immunosorbent assay using a Lazurite Automated ELISA System instrument. Thermo Scientific ELISA kits were used to assess cytokine levels.

The digital material of all experiments was statistically processed using non-parametric analysis methods.

3 Results and their discussion

The results of the study of sinusoidal cell dynamics in normal and in the background of loading with plant extracts under CCl₄-induced oxidative stress are presented in Figure 1.

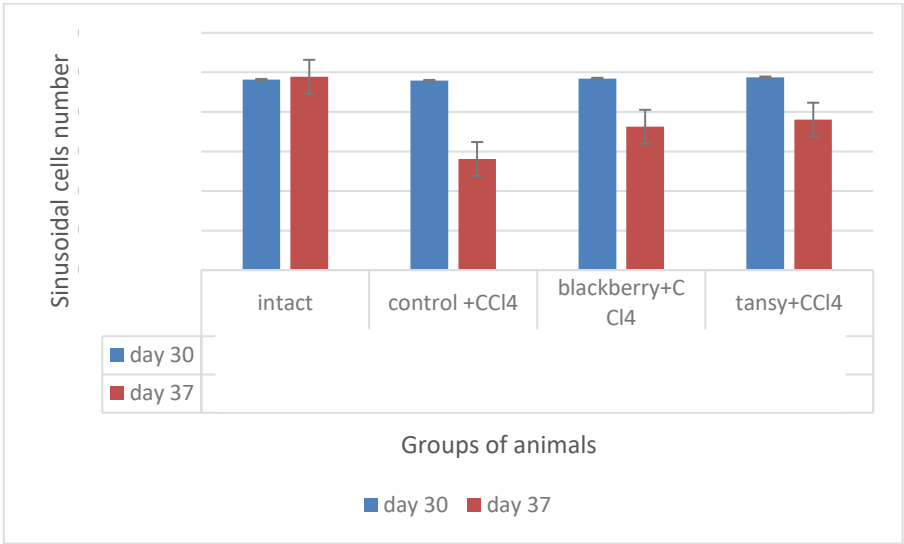


Fig.1. Dynamics of sinusoidal cells in normal and plant extract loaded conditions during oxidative stress modelling

The data presented in the figure show that in intact animals the number of sinusoidal cells in liver tissues was practically unchanged, whereas against the background of CCl4 injection into the organism in the form of an oil solution, a decrease in their number by 41.3% ($U=157.8000$, $Z=-2.974471$ at $p=0.000011$) from the initial value and by 42.5% ($U=187.2000$, $Z=-3.684471$ at $p=0.000017$) in relation to the indicators of intact animals on the 37th day was detected. A similar trend was observed in the experimental groups of rats: the number of sinusoidal cells in the liver tissues of rats receiving blackberry extract as an additional load decreased by 25.2% ($U=101.80000$, $Z=-3.596625$ at $p=0.0$) in comparison with the initial value and by 25.9 % ($U=181.60000$, $Z=-4.152254$ at $p=0.000221$) in comparison with intact animals; in rats treated with tansy extract - decreased by 21.9 % ($U=191.9000$, $Z=-2.522711$ at $p=0.0$) in comparison with the initial value and by 22.2 % ($U=144.40000$, $Z=-2.392214$ at $p=0.0$) in comparison with intact animals. It should be noted that the number of sinusoidal cells in the liver tissues of rats receiving plant extracts was higher than in the control group with induced oxidative stress: in rats receiving blackberry extract - by 11.7% ($U=177.7000$, $Z=-3.822171$ at $p=0.0$), in animals receiving tansy extract - by 35.4% ($U=201.5000$, $Z=-2.933141$ at $p=0.0$).

The results of the cytokine concentration study in the liver tissues of experimental animals in norm, against the background of antioxidant loading under induced oxidative stress, are presented in Table 1.

Table 1. Concentration of cytokines in liver tissues of rats in norm and against the background of antioxidant stress under induced oxidative stress

Group	Cytokines						
	IL-1α, pg/ml	IL-6, pg/ml	IL-18, pg/ml	TNFα, pg/ml	IFNγ, pg/ml	IL-10, pg/ml	TGF-β, pg/ml
1	951,25±34,25	28,31±0,88	562,31±20,23	688,18±24,78	951,31±30,44	180,86±6,51	822,25±29,60
2	1493,62±53,77 ¹	37,93±1,17 ¹	783,43±28,21 ¹	1162,13±37,19 ¹	729,27±23,34 ¹	512,37±16,9 ¹	239,41±8,37 ¹
3	1295,84±57,38 ^{1,2}	35,61±1,13 ¹	703,21±22,51 ^{1,2}	1039,22±33,25 ^{1,2}	813,61±26,04 ^{1,2}	380,59±12,55 ^{1,2}	621,29±21,75 ^{1,2}

4	1212,36±61,2 8 ^{1,2}	36,30±1,3 1 ¹	691,45±23,5 2 ^{1,2}	997,81±31,93 ¹ .2	851,14±27,2 4 ^{1,2}	336,44±11,7 7 ^{1,2}	579,34±18,5 4 ^{1,2}
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Note. In this table, the differences are reliable at $P<0.05$: 1 - in comparison with the indices of intact animals; 2 - in comparison with the indices of the control group of rats with induced oxidative stress.

In the liver tissues of rats in group 2 an increase in the concentration of IL-1 α by 57.0% (U =122.300, Z = -3.475111 at P=0.000293) was found on day 37 of the experiment in comparison with the first group. The liver tissues of group 3 rats also showed an increase in IL-1 α concentration of 36.2% (U =147.7000, Z = -3.154411 at p =0.000374) compared to group 1 animals, while it was 13.2% less (U =154.10000, Z = -2.9655512 at p =0.000217) compared to group 2 animals. In the liver tissue of group 4 rats, a similar trend was observed - an increase in IL-1 α concentration of 27.5% (U =117.2000, Z = -3.547444 at p =0.000000) was found compared to group 1 animals and at the same time it was 18.8% less (U =168.1000, Z = -3.54111111 at p =0.000251) compared to group 2 animals.

The concentration of IL-6 in the liver tissue of rats in group 2 was 34.0% higher (U =172.6000, Z = -3.582221 at p =0.0) on day 37 of the experiment compared to animals in group 1. In the liver tissue of rats in group 3, the concentration of IL-6 was found to be 25.8% higher (U =131.5000, Z = -2.41222221 at p =0.000000) compared to the animals in group 1 and at the same time 6.1% lower compared to the values in group 2. A similar trend was observed in the liver tissues of rats in group 4 - the concentration of IL-6 was 28.2% (U =147.6000, Z = -3.511733 at p =0.003441) higher than in animals in group 1 and 4.3% lower than in rats in group 2.

The concentration of IL-18 in the liver tissue of the rats in group 2 was 39.3% (U =121.2000, Z = -3.362221 at p =0.000415) higher than in the intact animals on day 37 of the experiment. In the liver tissues of rats in group 3, an increase in IL-18 concentration of 25.1% (U =164.30000, Z = -2.937741 at p =0.000215) was found compared to the animals in group 1 and at the same time it was 10.2% lower (U =177.30000, Z = -4.569912 at p =0.000001) compared to the parameters of group 2. A similar trend was observed in the liver tissues of group 4 rats - an increase in IL-18 concentration of 22.9% (U =136.6000, Z = -3.844741 at p =0.002495) was found in comparison with group 1 animals, but at the same time the IL-18 concentration was 11.7% (U =161.1000, Z = -2.921114 at p =0.000012) lower than the indicators of group 2.

In the liver tissue of the rats in group 2, an increase in the concentration of TNF α of 68.9% (U =201.7000, Z = -2.694117 at p =0.000217) was found on day 37 of the experiment compared to the animals in the first group. The liver tissue of rats in the third group also showed an increase in TNF α concentration of 51.0% (U =155.7000, Z = -3.463251 at p =0.000119) compared to the animals in the first group and at the same time it was 10.6% lower (U =114.4000, Z = -4.176621 at p =0.000017) compared to the animals in the second group. A similar trend was observed in the liver tissues of group 4 rats - an increase in TNF α concentration of 44.9% (U =201.50000, Z = -3.561141 at p =0.000421) was found compared to group 1 animals and at the same time it was 14.1% less (U =133.3000, Z = -2.726221 at p =0.000001) compared to group 2 animals.

The concentration of IFN γ in the liver tissue of the rats in group 2 on day 37 of the experiment was 23.3% lower (U =191.5000, Z = -3.533222 at p =0.000019) compared to the values of the animals of group 1. In the liver tissue of rats in group 3, the IFN γ concentration was 14.5% lower (U =172.8000, Z = -3.782221 at p =0.000227) compared to the animals in group 1 and at the same time 11.6% higher (U =196.3000, Z = -4.371114 at p =0.000026) compared to the indicators of group 2. A similar trend was observed in the liver tissues of rats in group 4, with a 10.5% decrease in IFN γ concentration (U =107.3000, Z = -3.752221 at p =0.003333) compared to animals in the first group and was 16.7% (U =128.6000, Z = -4.163312 at p =0.000184) higher than that of the rats in the second group.

In the liver tissues of the rats of the second group, a 2.8-fold increase in the concentration of IL-10 ($U=139.2000$, $Z=-3.496339$ at $p=0.000013$) was found on day 37 compared to the intact animals. The liver tissue of group 3 rats also showed a 2.1-fold increase in IL-10 concentration ($U=191.2000$, $Z=-3.496332$ at $p=0.000011$) compared to group 1 animals and at the same time it was 25.7% lower ($U=107.9000$, $Z=-3.322622$ at $p=0.000297$) compared to group 2 animals. A similar trend was observed in the liver tissues of group 4 rats - the concentration of IL-10 was 1.9 times higher ($U=122.8000$, $Z=-3.496332$ at $p=0.000321$) compared to the animals of the first group and at the same time it was 34.3% lower ($U=119.3000$, $Z=-4.396666$ at $p=0.000022$) compared to the indicators of the second group.

The concentration of TGF- β in the liver tissue of the rats in the second group on the 37th day of the experiment was 3.4 times lower ($U=149.1000$, $Z=-3.633333$ at $p=0.000333$) compared to the parameters of the animals in the first group. In the liver tissues of rats in group 3, the concentration of TGF- β decreased by 24.4% ($U=193.3000$, $Z=-3.196621$ at $p=0.000000$) in comparison with the animals of the first group and at the same time it was 2.6 times more ($U=181.2000$, $Z=-3.732221$ at $p=0.000001$) in comparison with the indicators of the second group. In liver tissues of rats in group 4 a similar tendency was observed - decrease of TGF- β concentration by 29.5% ($U=129.6000$, $Z=-3.233222$ at $p=0.002221$) in comparison with animals of the first group and at the same time it was 2.4 times ($U=191.8000$, $Z=-3.611711$ at $p=0.002633$) higher than indices of rats of the second group.

The results of the study of cytokine concentration in the blood plasma of the experimental animals in norm, against the background of antioxidant loading under induced oxidative stress are presented in Table 2.

Table 2. Concentration of cytokines in blood plasma of rats in norm and against the background of loading with plant extracts under induced oxidative stress

Group	Cytokines			
	TNF α , pg/ml	IL-6, pg/ml	IL-18, pg/ml	IL-10, pg/ml
1	16,29 $\pm$ 0,59	40,19 $\pm$ 1,41	5,61 $\pm$ 0,21	3589,13 $\pm$ 122,0
2	571,36 $\pm$ 18,28 ¹	42,44 $\pm$ 1,51	8,42 $\pm$ 0,29 ¹	89,58 $\pm$ 2,93 ¹
3	486,41 $\pm$ 17,15 ^{1,2}	51,28 $\pm$ 1,99 ^{1,2}	8,03 $\pm$ 0,33 ¹	829,32 $\pm$ 30,68 ^{1,2}
4	493,61 $\pm$ 17,29 ^{1,2}	59,31 $\pm$ 1,85 ^{1,2}	8,17 $\pm$ 0,27 ¹	993,69 $\pm$ 35,21 ^{1,2}

Note. In this table, the differences are reliable at $P<0.05$: 1 - compared with the indices of intact animals; 2 - compared with the indices of the control group of rats with induced oxidative stress.

In the blood plasma of the rats in the second group an increase of the concentration of TNF α by 35,1 times ($U=113,100$, $Z=-2,547741$ at $P=0,000013$) was found on the 37th day of the experiment in comparison with the animals in the first group, which indicates toxic liver damage by tetrachloromethane and intensive oxidative stress. The plasma of group 3 rats also showed a 29.9-fold increase in TNF α concentration ($U=191.3000$, $Z=-3.844711$ at $p=0.000001$) compared to the animals in the first group, and at the same time it was 14.8% lower ($U=161.1000$, $Z=-2.744141$ at $p=0.000291$) compared to the animals in the second group. A similar trend was observed in the plasma of group 4 rats - the increase in TNF α concentration was 30.3 times higher ($U=197.8000$, $Z=-3.147111$ at $p=0.000119$) compared to group 1 animals and at the same time it was 13.6% lower ($U=125.6000$, $Z=-2.966744$ at $p=0.000341$) compared to group 2 animals.

The concentration of IL-6 in the plasma of group 2 rats was insignificantly higher than that of group 1 animals on the 37th day of the experiment. In the plasma of group 3 rats, an increase of 27.5% ($U=154.1000$, $Z=-2.726622$ at $p=0.000214$) in IL-6 concentration was

found in comparison to group 1 animals and an increase of 26.1% ($U = 112.20000$, $Z = -2.463332$ at $p = 0.003333$) in comparison to group 2 animals. A similar trend was observed in the blood plasma of group 4 rats - an increase in IL-6 concentration of 47.5% ($U = 181.7000$, $Z = -4.314447$ at $p = 0.002144$) compared to group 1 animals and an increase of 39.8% ($U = 137.5000$, $Z = -2.641117$ at $p = 0.003141$) compared to group 2 animals.

The concentration of IL-18 in the plasma of group 2 rats was 50.1% ($U = 139.8000$, $Z = -2.647711$ at $p = 0.000000$) higher than in group 1 animals on 37 days of the experiment. The plasma of group 3 rats showed an increase in IL-18 concentration of 43.1% ($U = 141.10000$, $Z = -3.647711$ at $p = 0.000371$) compared to group 1 animals and a decrease of 4.6% compared to group 2 animals. A similar trend was observed in the plasma of group 4 rats, with a 45.6% increase in IL-18 levels ($U = 167.1000$, $Z = -2.921111$ at $p = 0.003111$) compared to group 1 animals, but IL-18 levels comparable to group 2 animals.

In the blood plasma of group 2 rats compared to group 1 animals, a 40.1-fold decrease in IL-10 concentration ($U = 129.8000$, $Z = -3.697111$ at $p = 0.000119$) was found on day 37 of the experiment. The plasma of group 3 rats also showed a 4.3-fold decrease in IL-10 concentration ($U = 151.1000$, $Z = -4.523332$ at $p = 0.000114$) compared to group 1 animals and a 9.2-fold increase ($MaU = 147.5000$, $Z = -3.723361$ at $p = 0.000118$) compared to group 2 animals. A similar trend was observed in the blood plasma of group 4 rats, with a 3.6-fold decrease in IL-10 concentration ($U = 191.1000$, $Z = -3.385541$ at $p = 0.002223$) compared to group 1 animals and an 11.1-fold increase ($U = 139.6000$, $Z = -3.347114$ at $p = 0.000001$) compared to group 2 animals.

According to the results of the experiment, it was found that the number of sinusoidal cells in liver tissues decreases with toxic liver damage caused by tetrachloromethane, but preliminary loading of rats for 30 days with aqueous extracts of tansy and blackberry and parallel loading with CCl₄ for 6 days did not promote such a sharp decrease in the number of sinusoidal cells in liver tissues as in rats of the control group.

In the case of oxidative stress in blood plasma, there is increased production of the pro-inflammatory cytokine TNF α and suppression of IL-10 production, while the concentration of the pro-inflammatory cytokines IL-6 and IL-18 does not change significantly. At the same time, in response to diffuse toxic liver damage and oxidative stress in liver tissue, the concentration of cytokines IL-1 α , IL-18, TNF α , IL-10 increases and the concentration of IL-6, IFN γ , TGF- β decreases. Increased production of IL-1 α and IL-18 in liver tissue seems to trigger local inflammation by increasing the levels of cytokines such as TNF α , IL-6. The increased concentration of the anti-inflammatory cytokine IL-10 confirms the key role of this mediator in the regulation of the immune response and its ability to suppress the secretion of the pro-inflammatory cytokines TNF α and IL-6.

4 Conclusion

Aqueous extracts of fir and blackberry modulate to different degrees the functional state of sinusoidal cells in early periods of toxic exposure to tetrachloromethane, which contributes to the early resolution of the inflammatory process. Exposure of aqueous extracts of fir and blackberry to sinusoidal cells changes the production of regulatory factors, which compensates the speed of recovery processes after toxic exposure.

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