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Hepatoprotective Action of Boric Mineral Waters in Toxic Hepatosis: Experimental Study

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Abstract

Chronic alcoholic hepatosis is one of the most common liver diseases. The intake of alcohol leads to overstrain of enzyme systems in the liver, which, in turn, causes a cascade of subsequent metabolic disorders. A wide range of medicinal substances is used to correct the results of injuries in the hepatobiliary system. In general, these are pharmacochemical preparations, however, recently researchers have begun to pay more attention to compounds of natural origin. There is an extensive group of therapeutic factors - mineral waters (MW), which have a powerful biological, including detoxification effect practically without contraindications. In particular, the results of studies have revealed the stimulating effect of MW on the repair processes in the liver. In an experiment on white rats with a model of toxic hepatosis (TH) was studied the effect of boric mineral waters (MW) with different osmolarity and content of boron. Four experimental groups were formed. The 1st group (control group) was made up of intact animals. In the rats of the 2nd, 3rd and 4th groups, the TH model was reproduced by daily injection to rats of a 25% aqueous solution of ethanol for 45 days. After achievement of clinically and biochemically confirmed model of TH, correction of violations of the functional state of the liver was performed. Rats of the 3rd and 4th groups received two medium-mineralized hydrocarbonate chloride-sodium waters with different osmolarity and content of boron. The duration of the MW course was 30 days. Antitoxic function of the liver was determined using a thiopental test. Functional state of the liver was determined by the activity of the enzymes - alanineaminotransferase (AlT), aspartateaminotransferase (AsT) and the level of total bilirubin and its fractions in the serum. Morphological studies were carried out for determining the structural changes in liver tissue. It has been established that the method used for the use of these MW can significantly reduce the manifestations of chronic alcoholization. The use of boric MW causes the restoration of the structural organization of liver of rats with hepatosis. The influence of boric MW on the detoxification function of the liver is also indicated by a change in the situation with the content and composition of bilirubin.

Keywords

Toxic Hepatosis, Boric Mineral Water, Liver

1. Introduction

Along, regular use of alcoholic beverages by the population is currently an urgent medical and social problem [1-3]. Despite a large number of studies devoted to the study of the mechanisms of the biological action of ethanol, many

aspects of its influence on the human body remain not fully understood [4]. The liver is one of the first organs that suffer with oral administration of xenobiotics, including ethanol. Structural and functional changes in the liver during alcoholization have been investigated by many authors [5], [6]. However, since alcoholic lesions of the hepatobiliary system and associated loss of ability to work and disability of the population do not decrease, the rehabilitation of these lesions remains a serious national problem [7]. The intake of alcohol leads to overstrain of enzyme systems in the liver, which, in turn, causes a cascade of subsequent metabolic disorders.

The first of the intermediate products of alcohol metabolism, formed in the liver, is acetaldehyde, the toxicity of which is 30 times higher than the toxicity of ethyl alcohol. Its appearance in the liver tissue stimulates the processes of lipid peroxidation with the formation of free radicals, which cause a whole complex of damages - the destruction of membrane phospholipids, fragmentation of nucleic acids [8]. In turn, the disruption of the nucleic acid metabolism stimulates the synthesis and accumulation of alcoholic hyaline, which causes the development of autoimmune reactions, including hyperproduction of anti-inflammatory cytokines, which creates an additional burden on the immune system.

Damage to the cellular membranes of hepatocytes due to a decrease in the content of phosphatidylcholine affects the ultrastructure of the mitochondria, as a result of which the absorption of oxygen decreases and energy formation decreases [9]. To correct the results of alcohol damage to the hepatobiliary system, a wide range of medicinal substances possessing protective properties is used. In general, these are pharmacochemical preparations, however, recently researchers have begun to pay more attention to compounds of natural origin. [10].

Among the latter, there is an extensive group of therapeutic factors - mineral waters (MW), which have a powerful biological, including detoxification effect practically without contraindications (unlike medicinal products) [11]. In the liver, MW changes enzyme activity and improves its functional state. In particular, the results of studies have revealed the stimulating effect of MW on the repair processes in the liver [12]. The positive effect of the action of MW in their internal application is associated with the peculiarities of their physicochemical composition: the presence of biological agents, features of the ionic composition, different osmolality and other characteristics [13, 14]. Our previous studies on healthy white rats with using the MW with different osmolarity and different boron content have established a unidirectional (but different intensity) stimulating effect on the detoxification function of the liver (on the rate of biotransformation of barbiturates), activation of the processes of bile formation and biliary excretion. It has also been found that the process of urination increases due to an increase in the glomerular filtration rate in nephrons, and activation of the excretory function of the kidneys by increasing the excretion of creatinine, urea and chloride ions with 24-hour urine [15].

Proceeding from the foregoing, the possible prospective solution of the problem of hepatoprotection in toxic hepatosis (TH) can be the use of boric MW.

2. Materials and Methods

The aim of the study - the structural and functional changes in the liver of rats caused by toxic hepatosis of different durations, and the possibility of correcting these disorders by internal dosed application of boric mineral waters.

The materials of the present study were the results obtained from a complex study of the course effect of two boric MW on white rats in the Wistar line of autobreed reproduction of rats. Experimental work with animals was conducted in accordance with the recommendations and rules which governing this activity [16-18]. In accordance with the tasks of the work, rats were ranked into 4 groups. In each group, there were 15 rats. The 1st group (control group) was made up of intact animals. In the rats of the 2 nd, 3 rd and 4 th groups, the TH model was reproduced.

The chronic toxic hepatosis model was reproduced by daily injecting a 25% aqueous ethanol solution at a dose of 1.5% of body weight (based on 4 g of 96% ethanol per kg) daily for rats for 45 days [19]. A solution of ethanol was administered to rats of the second, third and fourth groups daily, at 13 hours, in a gullet with a soft probe with olive.

After achievement of clinically and biochemically confirmed model of TH, correction of violations of the functional state of the liver was performed. Rats of the 3rd and 4th groups received two medium-mineralized hydrocarbonate chloride-sodium drinks of water with different osmolarity and content of boron. The rats of groups 3 and 4 were given daily appropriate degassed MW in an amount of 1% of body weight.

Rats of the 3rd group was received MW "Luzhanskaya". "Luzhanskaya" is a medium-mineralized bicarbonate chloride-sodium water. Formulas for the chemical composition of this MW:

$$H^3BO^3O_{74.41}M_{4.71}\frac{HCO_396Cl4}{(Na+K)88Cal1Mg1}$$

The concentration of H_3BO_3O is 74.0 mg/l, the total mineralization is 4.71 g /l, the osmolarity is 99.3 mosm/l.

Rats of the 3rd group were received MW «Polyana Kvasova». «Polyana Kvasova» is a medium-mineralized bicarbonate chloride-sodium water. Formulas for the chemical composition of this MW:

$$H^3BO^3O_{212.96}M_{10.22}\frac{HCO_390Cl10}{(Na+K)96Cal4Mg1}$$

The concentration of H_3BO_3O is 212.96 mg/l, the total mineralization is 10.22 g/l, the osmolarity is 228.5 mosm/l.

The duration of the MW course was 30 days. The duration

of the experiment was 45 days. In the course of the experiment, the study material was taken 16-18 hours after the last MW injection. For a comprehensive assessment of the structural and functional state of the liver, we determined: the state of the antitoxic function of the liver with the help of a thiopental sample. The functional state of the liver was determined by the activity of the enzymes - alanineaminotransferase (AlT), aspartateaminotransferase (AsT) in the blood serum by the Reitman-Frenkel method (a set of Filicite), the level of total bilirubin and its fractions in the serum by the method of Jendraschek, Cleggorn, and Grof. Morphological studies determined structural changes in liver tissue, wherefore, in a rat, a portion of the liver was withdrawn in a volume of $1cm^3$ and was carried through the spirits of increasing concentration and poured into celloidin. Histological cuts were made, which stained hematoxylin-eosin. Microscopic examination of the liver was performed on the sections obtained. The obtained data were compared with similar parameters of the functional status of the liver of the rats of the comparison group and the control group.

The detoxification function of the liver was examined using a Speransky thiopental test, which allows for the duration of the sleep of the animals caused by the effect of anesthesia to estimate the rate of metabolism of the thiopental, which is carried out by the cytochrome P-450-dependent monooxygenase system of hepatocytes, and characterizes the state of one of the leading functions of the liver - antitoxic. The definition and evaluation of indicators of the structural and functional characteristics of the liver were carried out in accordance with approved methods [20], [21]. The statistical processing of the results was carried out by conventional statistical methods using the Student's t-test [22].

3. Results and Discussion

According to the data of table 1, the development of chronic toxic hepatosis (45 days) in rats is accompanied by inactivation of the detoxification function of the liver, as evidenced by a significant lengthening of the medication sleep time ($P < 0.001$). Other functions of the liver are also disturbed. In rats of the second group, the activity of AlT and AsT decreases, while the decrease in activity occurs in an uneven degree, this causes an increase of the Rytis index, which indicates the presence of dystrophic processes in hepatocytes.

The development of dystrophic changes in the liver of rats with prolonged alcoholization is confirmed by data from histological studies (Figure 1).

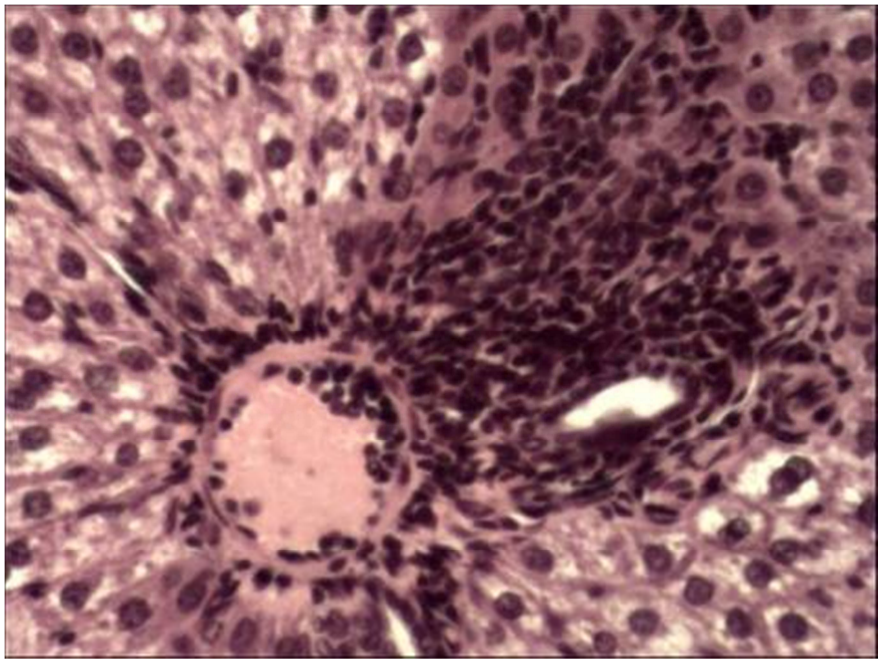

Figure 1. *Liver of rat after 45 days of alcoholization (II group). Coloring of hematoxylin-eosin. Magnification: x 200.*

These studies have revealed that the lobulation of the hepatic tissue and the ordering of the location of hepatocytes in the beams are preserved. However, the size of the hepatocytes is not the same, especially it is expressed more closely to the periphery of the lobules, therefore in this part of the lobules the location of the hepatocytes is disordered. Part of normal hepatocytes, in the center of them is a medium-sized dense nucleus, a homogeneous pale basophilic cytoplasm. Part of hepatocytes with a small nucleus, in the cytoplasm they have vacuoles in different amounts. There are more such cells in the zone adjacent to the central vein. Clusters of lobules around the vessels of the triad have an accumulation of lymphocytes. Intermediate spaces are slit-shaped. The development of dystrophic processes in the liver is due to inactivation of its detoxification function, which contributes to the accumulation of toxic metabolites in the rat's body. Confirmation of this is the data on the change in the ratio of bilirubin fractions, given in the table. Indirect

bilirubin in the blood of healthy rats is contained in 1.75 times more than direct, that is, in normal detoxification activity of the liver provides disinfection of most of the toxic product of hemoglobin metabolism. In conditions of chronic alcoholization, the ratio of direct and indirect bilirubin changes, the latter becomes 3.5 times more than direct, that is, alcoholization leads to inhibition of the detoxification function of the liver and the accumulation of a toxic metabolite, which is also neurotoxic.

The use of boric MW in experimental animals with TH significantly altered the functional state of the liver. According to the data of table 1, the use of these MW significantly increased the time of falling asleep in relation to the data of intact animals and rats with eliminated alcoholization. At the same time, the time of drug sleep was reliably reduced in animals receiving both MW, however, the phenomenon revealed is more pronounced when receiving rats MW «Polyana Kvasova», which can be associated with both restoration of the detoxification function of the liver and the level of activity of the autonomic nervous system ($P<0.05$ and $P<0.001$, respectively).

Table 1. *Characteristics of indicators of the functional state of the liver of rats.*

Indicators	I GROUP	II group		III group		IV group	
	($M_1 \pm M_1$)	($M_2 \pm m_2$)	P_2	($M_3 \pm m_3$)	P_3	($M_4 \pm m_4$)	P_4
Duration of drug sleep, min.	53.40 ± 1.26	59.11±1.10	< 0.01	50.12 ± 1.14	>0.05	45.07 ± 1.23	< 0.01
AlT, mkmol/см^3	113.31 ± 2.13	83.56 ± 3.67	< 0.01	77.57 ± 2.67	< 0.01	88.91 ± 4.98	< 0.01
AsT, mkmol/см^3	289.64±1.12	267.43±5.40	< 0.01	219.29 ± 4.13	< 0.001	218.85±3.15	< 0.01
Ritis indeks, c.u	2.56 ± 0.11	3.22 ± 0.14	< 0.01	2.80 ± 0.56	> 0.05	2.42 ± 0.45	> 0.05
Bilirubin, mkmol/l							
common	8.44 ± 0.28	5.48 ± 0.44	< 0.01	7.53 ± 1.15	> 0.05	3.79 ± 0.35	< 0.01
direct	3.06 ± 0.18	1.21 ± 0.12	< 0.01	1.55 ± 0.54	< 0.05	1.19 ± 0.12	< 0.01
indirect	5.38 ± 0.15	4.27 ± 0.35	< 0.05	5.98 ± 1.40	> 0.05	2.59 ± 0.34	< 0.01

Note. P_2, P_3, P_4 - reliability of the differences between the indices of the respective group and the control

As for the status of other liver functions, according to the table 1, radical changes in the activity of AlT and AsT do not occur in relation to the rats of group 2, since the activity of these enzymes remains lower than in intact animals. However, a significant decrease in the activity of AsT in the application of MB «Luzhanskaya» and «Polyana Kvasova» causes a decrease in the Ritis index to the level of intact animals, that is, it can be assumed that the use of both MB containing boron inactivates dystrophic processes in hepatocytes, but more this effect is expressed in animals that received MW «Polyana Kvasova».

Histological studies of the liver of rats receiving MW «Luzhanskaya» showed no special changes in the microscopic pattern with respect to rats with TH (Figure 2)

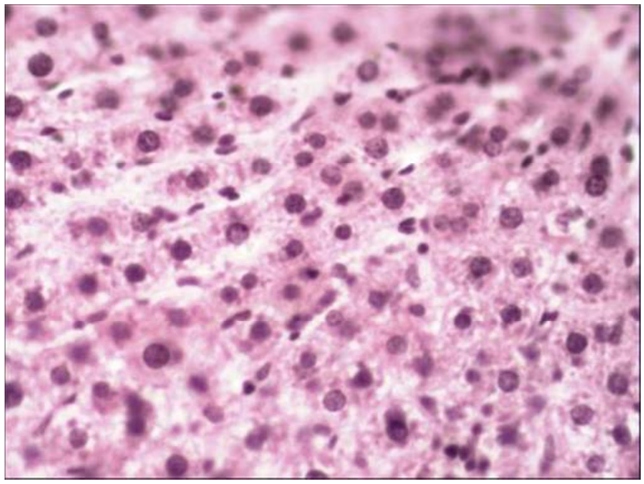

Figure 2. *Liver of a rat with chronic alcoholic intoxication, which used MW "Luzhanskaya" for 30 days (III group). Coloring: hematoxylin-eosin. Increase: x 200.*

Preserved lobulation, there are cells with vacuolization of the cytoplasm. A feature of liver cells in the rats of this group was the presence of nuclei with a clear granular-fibrous pattern of chromatin and eosinophilia of the cytoplasm. In addition, inter-beam spaces are somewhat expanded. Histological studies of the liver of rats treated with MW «Polyana Kvasova» did not reveal hepatocytes with vacuolization of the cytoplasm (Figure 3).

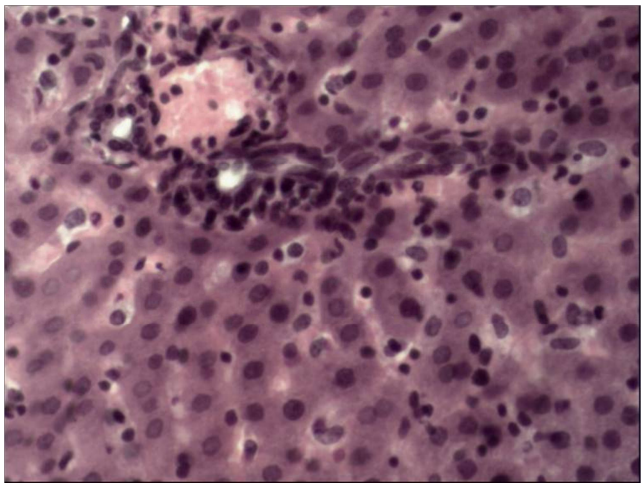

Figure 3. *Liver of a rat with chronic alcohol intoxication, which was used MW "Polyana Kvasova" (IV group). Hepatocytes with enlarged nuclei. Coloring: hematoxylin-eosin. Increment: x 400.*

Clusters of lymphocytes do not remain near the vessels of the triads. As for the hepatocytes themselves, they are characterized by a granular eosinophilic cytoplasm and lightened nuclei with a clear pattern of chromatin. In other words, the use of MW «Polyana Kvasova» causes the restoration of the structural organization of liver of rats with

hepatosis. The influence of boric MW on the detoxification function of the liver is also indicated by a change in the situation with the content and composition of bilirubin.

According to the data of table 1, against the backdrop of MW "Luzhanskaya" application the amount of total bilirubin approaches the norm, that is, bile formation is increased in comparison with animals with eliminated alcoholization. At the same time, the ratio of indirect/direct bilirubin is 3.85, that is, the prevalence of toxic bilirubin corresponds to alcoholized rats and this indicates the preservation of the depressed state of detoxification of the liver.

These data are in good agreement with the dystrophic changes in the liver that were preserved in rats who received MW "Luzhanskaya". Observed in rats receiving MW «Polyana Kvasova», a further decrease in the content of total bilirubin indicates the inhibition of bile formation, which agrees with the known action of boron, which in this MV is greater than in Luzhanskaya [16]. In this case, the ratio of indirect/direct bilirubin is 2.71, which is higher than in intact rats, but less than in the experimental groups. It can be assumed that the inhibition of bile formation contributes to the enhanced detoxification activity of hepatocytes.

4. Conclusion

Thus, studies have shown that prolonged alcoholization of rats is accompanied by the formation of dystrophic changes in the liver and a violation of its detoxification function. The use of boric mineral waters, depending on the osmolarity and boron concentration, reduces to some extent the dystrophic changes in hepatocytes and positively affects the detoxification function of the liver. These changes in the liver of animals, in turn, can have a positive effect on the functional activity of other organs and systems, including the state of the central nervous system.

The results of the experimental studies scientifically justify the use of boric MW separately (taking into account the nature of biological activity) or in combination with other therapeutic factors, to correct the manifestations of chronic toxic hepatosis caused by prolonged alcoholization.

Conflict of Interest Statement

All the authors do not have any possible conflicts of interest.

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