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REPORT:

**EFFICACY OF APPLICATION OF THE BIOLOGICALLY ACTIVE
SUPPLEMENT “WATER SOLUTION OF HYDRATED C₆₀ FULLERENE”
IN COMPLEX THERAPY OF THE PATIENTS WITH CHRONIC
HEPATITIS OF TOXIC GENESIS**

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ОТЧЕТ

**Эффективность применения биологически активной добавки
«водный раствор гидратированного фуллерена C₆₀» в
комплексной терапии больных хроническими гепатитами
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INTRODUCTION

Urgency of the subject.

Chronic hepatitis (CH) is one of the urgent problems of modern hepatology. CH has a great social significance, connected with high rate of the disease, mainly among working-age population, with significant percentage of complicated and fatal outcomes [9, 13, 26, 33]. By no means always conventional methods of treatment turn to be effective and prevent adverse outcomes. At the same time, the potential of world's medical science enables to determine key links of the pathogenesis of chronic hepatitis and liver cirrhosis, which promotes the development of new methods of diagnostics and treatment.

Modern theories of pathogenesis of chronic hepatitis and liver cirrhosis formation are based on such aspects as influence of external factors, responsible for disease initiation, and mechanisms of individual's response, which determinate the reaction of the organism. And the leading role belongs to immunological mechanisms [2, 14, 17]. The development of immunological reactions is associated with main ecological factors, such as viruses, alcohol, hepatotoxines, which promote the disturbance of structure and parenchyma function of the liver, regeneration processes of connective tissue components [19, 20, 29].

Nowadays in literature there are many works on the results of use of biologically active supplements (BAS) in healthy people. But the issue of BAS influence on the functional state of the liver in chronic hepatitis patients is still not studied enough.

Pre-clinical study of the biologically active supplement "water solution of hydrated C₆₀ fullerene" demonstrated the absence of acute and subchronic toxicity at its use. There was also revealed a number of specific activities of fullerene in animal experiments, including anti-inflammatory, regenerative, membrane stabilizing and adaptogenic, which can serve as a basis for development of new therapeutic strategy at use of conventional therapy of chronic hepatitis of toxic

genesis in combination with fullerene [5]. Possibly, the indicated approach of treatment of CH patients can improve the effectiveness of therapy of this patient category.

In this connection, the study of complex therapy of chronic hepatitis with use of biologically active supplements is reasonable.

Objective and tasks of the study:

The objective of this work is to increase the effectiveness of the treatment of chronic hepatitis patients with use of biologically active supplement “water solution of hydrated C₆₀ fullerene ” in complex therapy.

To achieve this objective the following tasks should be solved:

1. To determine the influence of the treatment with BAS “water solution of hydrated C₆₀ fullerene” on clinical manifestations of CH.
2. To study the indexed of clinical analysis of blood in the examined CH patients under the influence of the proposed therapy with BAS “water solution of hydrated C₆₀ fullerene” in comparison with conventional therapy.
3. To study the influence of combined treatment with BAS “water solution of hydrated C₆₀ fullerene” on enzyme exchange in patients with chronic hepatitis of toxic genesis.
4. To study the influence of combined treatment with BAS “water solution of hydrated C₆₀ fullerene” on pageantry exchange in patients with chronic hepatitis of toxic genesis.
5. To study the influence of combined treatment with BAS “water solution of hydrated C₆₀ fullerene” on lipid exchange in patients with chronic hepatitis of toxic genesis.
6. To study the influence of combined treatment with BAS “water solution of hydrated C₆₀ fullerene” on the state of lipid peroxidation (LPO) and antioxidant protection (AOP) in patients with chronic hepatitis of toxic genesis.

Object of the study.

Patients with chronic hepatitis of moderate activity of toxic genesis.

Subject of the study.

Effectiveness of BAS “water solution of hydrated C₆₀ fullerene” in complex therapy of patients with chronic hepatitis of toxic genesis.

Methods of the study.

To verify the diagnosis of chronic hepatitis there were used clinical, biochemical and ultrasound research methods. The functional state of the liver was assessed with determination of whole protein and its fractions, total bilirubin and its fractions, the activity of transaminases, alkaline phosphatase, thymol test, the state of lipid peroxidation and antioxidative protection.

PART 1. MATERIALS AND METHODS OF THE STUDY

1.1. Clinical characteristics of the examined patients.

In the gastroenterological department of Kharkov regional clinical hospital, which is a base of Internal medicine department No 3 of KhNMU, there were examined 40 patients with chronic toxic hepatitis. The control group consisted of 20 practically healthy people.

Distribution of the patients as for their sex and age was done in accordance to International classification of age-specific periods. Among the patients there were 25 men and 15 women with the disease duration of 1 to 10 years. The majority of the patients were 62 individuals aged 45-59.

The diagnosis CH was formulated in accordance with the classification of chronic hepatitis (Los-Angeles, 1994). The diagnosis was verified on the basis of complains, anamnesis, clinical picture, and data of biochemical, immunological, roentgenologic, morphological and ultrasound investigations.

Forming clinical diagnosis much attention was paid to complains, anamnesis, clinical picture of the disease, as well as data of the laboratory and instrumental diagnostics.

Chronic hepatitis of toxic genesis in the examined patients was considered in case of the following signs:

- continuous alcoholic anamnesis, even if the patients were not registered as chronic alcoholics;
- absence of old acute viral hepatitis B (HBV), C (HCV) and D (HDV) (determination of hepatitis B, C, and D markers in the serum) in the anamnesis;
- absence of other old in diseases in the anamnesis: tuberculosis, syphilis, cholelithiasis, etc.;
- contact with hepatotropic poisons in household use or at work;
- long-term use of hepatotropic medicinal preparations.

Alcoholic anamnesis was collected from the patients, their relatives, district doctors and narcologists.

Alcoholic liver disease was established in 29 patients, and 11 patients were diagnosed with medicinal liver lesion. It is noted that alcoholic liver lesions were mainly found in men and medicinal ones – in women.

At objective examining of the patients with chronic hepatitis of toxic genesis there was estimated the frequency of symptoms, distinguishing the character of the pathological process in the liver.

In clinical picture of the examined CH patients more frequently were observed asthenovegetative (93.39 %), abdominal painful (80.16 %), dyspeptic (80.16 %) against the background of hepatomegaly (87.60 %).

Feverish, articular and encephalopathic syndromes were not represented significantly and constituted 26.4 %, 24 % and 45 %, correspondingly.

Abdominal painful syndrome manifested as sense of heaviness, pressure in the upper part of the abdomen.

Dyspeptic syndrome manifested as permanent nausea, increasing after the intake of food or medicinal preparations, eructation, loss of appetite, dryness and bitter taste in the mouth, vomiting, diarrhoea, and was observed in all groups of the examined patients, both of toxic and cryptogenic geneses.

Asthenovegetative syndrome manifested as indisposition, weakness, fatigability, performance decrement, psychoemotional instability, insomnia, weight loss, headaches, and was frequently observed in all groups of patients.

Hemorrhagic syndrome manifested as nasal and gingival hemorrhages, subcutaneous hemorrhage, and menorrhagias.

Cholestatic syndrome manifested as persistent or intermittent icteritiousness of cutaneous covering and itching.

Endocrine syndrome manifested mostly as gynecomastia.

Feverish syndrome manifested as prolonged low grade fever of the body.

Insignificant number of the patients had complaints about memory impairment, sleepiness, periods of time and spatial disorientation. This symptom complex was estimated as encephalopathic syndrome.

Pains in the right hypochondrium were noted in most patients. Large liver mass was observed in 40 CH patients, enlarged spleen – in 7 CH patients.

In order to make a more precise diagnosis of chronic hepatitis of toxic genesis the patients underwent complex laboratory and instrumental examination, allowing to work out the methods of complex therapy, aimed at the recovery of functional liver state.

2. Methods of patient examination

In order to achieve the objective and solve the tasks of the study there was conducted clinical and laboratory examination, including biochemical and ultrasound methods of investigation, which allow to estimate the functional state of the liver in patients with chronic hepatitis of toxic genesis.

Blood sampling for analysis was done in the morning on an empty stomach, the following day after patient's admission to hospital.

Method of analysis of whole serum protein.

For this work there was selected colorimetric biuretic method, fairly considered to be most specific, precise and practical [1].

Whole protein content in healthy people constituted on average 68.7 ± 0.2 g/l.

Method of analysis of serum protein spectrum.

In order to analyze serum protein fractions there was used the method of electrophoretic separation on paper, which is informative [1]. The content of protein fractions in healthy people constituted on average: albumins – 56.55 ± 0.98 %, alpha-1 globulins – 7.69 ± 1.14 %, alpha-2 globulins – 8.8 ± 1.98 %, beta-globulins – 11.09 ± 0.48 %, gamma-globulins – 14.61 ± 0.44 %.

Determination of total serum bilirubin and its fractions. Method of Jendrassik, Kleggorn and Grof provides the possibility of fractional determination of bilirubin content.

Total bilirubin in healthy people constituted 11.76 ± 0.64 $\mu\text{mol/l}$, conjugated bilirubin – 4.5 ± 1.13 $\mu\text{mol/l}$.

Method of analysis of enzymatic exchange.

To determine the activity of serum aminotransferase there are colorimetric and kinetic (spectrophotometric) methods. Colorimetric method, proposed by Reitman and Frankel, being technically simple, at the same time detects changes of enzymatic activity and gives reproducible results.

In healthy individuals the activity of aminotransferase on average constituted: AST – 0.43 ± 0.02 mmol/h·l, ALT – 0.38 ± 0.001 mmol/h·l.

Method of determination of serum gamma-glutamyl transpeptidase (GGT) activity.

For GGT determination there was used widespread nowadays unified method, developed at the Ukrainian research institute of gastroenterology (Dnepropetrovsk, 1995) with standard assay kit [1].

GGT content in healthy people constituted on average 1.75 ± 0.19 μ kat/l.

Method of determination of serum alkaline phosphatase (ALP). The most spread nowadays is the method of Bodansky, King and Armstrong. The used method is unified.

In control group of healthy individuals ALP activity was 0.65 ± 0.15 U.

Determination of thymol test. The determination was done by the method of Huergo and Popper. In healthy individuals the indexes of serum thymol test constituted 2.04 ± 0.01 U.

Determination of the content of primary LPO products.

In the study there was used the method of malonic dialdehyde (MDA) determination in serum and erythrocyte membranes by the method of M.S. Goncharenko and A.M. Latinovaya [7]

In healthy individuals MDA content in plasma constituted 0.62 ± 0.14 μ mol/l, in erythrocytes – 7.48 ± 0.63 μ mol/l.

The state of antioxidative protection was assessed by the indexed of peroxidase, catalase, ceruloplasmin, superoxide dismutase (SOD) in serum.

The determination of peroxidase activity was done according to the method of T.P. Popov and L.P. Neykova [15].

Peroxidase activity in healthy individuals constituted 280.35 ± 7.43 mmol/min \cdot l.

Determination of blood catalase was done by the method of Bach.

Blood catalase count in healthy individuals constituted on average – 16.36 ± 0.19 mg.

Determination of serum ceruloplasmin was done by modified method of Revin. Serum ceruloplasmin content in healthy individuals constituted 1.86 ± 0.13 $\mu\text{mol/l}$.

In order to **determine sulfhydic groups (SH-group) of protein and nonprotein compounds** there was used photolorimetric ultramicromethod of quantitative determination of V.F. Frolov [1]. In healthy individuals the content of total SH-groups constituted 2.24 ± 0.1 mmol/l , nonprotein SH-groups – 0.75 ± 0.11 mmol/l , protein SH-group – 1.49 ± 0.02 nmol/l .

When examining subject patients, there was used the method of ultrasonic echoscopy with ultrasonic echo chamber “Alloka – SSD – 630” (Japan). With this method there was studied topography, form, size, circuits and parenchyma of the liver, which allowed to make the diagnosis more precise.

Statistical processing of the results of the study was done with computer PC AMDK6 2/400 with the method of variation statistics, correlation, disperse and discrete-dynamic analysis. A program package, adapted for medical and biological investigations was used [12].

PART 2. RESULTS OF THE STUDY AND THEIR DISCUSSION

2.1. Comparative characteristic of the influence of conventional and proposed therapies on clinical manifestation of chronic hepatitis in patients

For determination of the effectiveness of the correcting therapy in dynamics there was studied functional liver state via studying protein, enzymatic and pigmentary exchange, the state of lipid peroxidation and indexes of antioxidative protection.

The study was carried out on day 2-3 after patient's admission to the clinic, as well as on day 21-23 after initiation of the treatment course.

Due to the type of therapy patients with CHMA of toxic genesis were divided into two groups: subgroup of patients 1– treated with conventional method, 2 – treated with proposed method.

The patients with CHMA of toxic genesis of group 1 along with dietotherapy (diet No 5 of Pevzner) received Essentiale-N 2 (600 µg) capsules 3 times a day before meals, as well as spasmolytics and enzymatic agent, as indicated. The second group of the patients received dietotherapy, Essentiale-N 2 capsules 3 times a day before meals, in combination with BAS “water solution of hydrated C₆₀ fullerene” 50 ml 3 times a day 15-20 min before meals for the first 3 days, then – 50 ml twice a day 15-20 min before meals for the first 3 days and for the following 15 days once a day 15-20 min before meals.

The basis for use of the proposed therapy with BAS “water solution of hydrated C₆₀ fullerene” is the results of pre-clinical studies.

The molecule of C₆₀ fullerene is a new allotropic form of carbon. Pre-clinical studies allowed to determine the following properties of fullerene: hepatoprotective, anti-inflammatory, regenerative, membrane stabilizing and adaptogenic [5]. Consequently, additional indication of biologically active supplement “water solution of hydrated C₆₀ fullerene” for patients with chronic hepatitis was aimed at improvement of the functional state of liver, immunologic reactivity and normalization of clinical and biochemical indexes of serum.

The assessment of effectiveness was carried out in each group as for dynamics of subjective sensations (the degree of manifestation of pain, dyspeptic, and asthenovegetative syndromes), objective data (size of liver, spleen, body weight, edema, and ascites), dynamics of laboratory, biochemical indexed, LOP and AOP state.

The analysis of clinical indexes of the examined patients with chronic hepatitis of moderate activity (CHMA) of toxic genesis revealed the following results.

There was observed subjective improvement and tendency to normalization of objective data with different degree of manifestation of the medicinal effect in subgroups.

As a result of the conducted treatment in patients (CHMA) positive dynamics was observed on day 13-14. The improvement was characterized by decreases of abdominal pain, dyspeptic, and asthenovegetative syndromes, tendency to normalization of the laboratory indexes, which was more expressed in the group of treatment with the proposed method.

Improvement of the subjective picture as decrease or elimination of abdominal pain syndrome (74%), decrease of dyspeptic (87%), asthenovegetative (83%), and icteric (62%) syndromes was observed by day 15-17 of the disease, and the improvement of the objective indexes was observed not until day 17-19 after the initiation of the treatment.

Hepatomegaly decreases was observed in 25 patients.

In the patients of the second group CHMA, receiving therapy with the use of BAS “water solution of hydrated C₆₀ fullerene ” there was observed more rapid decrease of asthenovegetative, dyspeptic, and, abdominal pain syndromes.

Positive dynamics of clinical symptoms is confirmed by the data of clinical and biochemical indexed, as well as LOP and AOS state.

2.2. Indexes of clinical blood analysis in patients with chronic hepatitis of toxic genesis under the influence of conventional and proposed therapy with fullerene

In CHMA patients there was observed tendency to leukocytosis, increased ESR, which reflected the presence of inflammatory processes in hepatocytes (Tabl. 2.1).

Table 2.1

Indexes of clinical blood analysis in the examined patients

Index	Control n=20	Group 1		Group 2	
		Before treatment n=20	After treatment with conventional method n=20	Before treatment n=20	After treatment with proposed method n=20
Hemoglobin, g/l	129.7±5.3	119.5±4.2	127.6±3.3*	118.9±4.1	129.6±5.1 */**
Erythrocytes, 10 ¹² /l	4.1±0.1	3.95±0.2	4.0±0.1*	3.95±0.2	4.0±0.1*/ **
CP	0.91±0.02	0.85±0.04	0.89±0.02*	0.85±0.04	0.91±0.03 */**
ESR, mm/h	6±2	20±2	17±2	21±2	10±2
Leukocytes, *10 ⁹ /l	4.7±0.8	10.2±0.8	11.1±0.9*	10.2±0.8	7.2±0.8**
Stab, %	4.8±0.5	6.2±0.4	7.1±0.5*	6.2±0.4	5.1±0.4*/ **
Segmentated, %	51.2±1.3	65.0±1.4	71.4±1.1*	65.0±1.4	55.4±1.3*/ **
Eosinophils, %	0.6±0.2	0.8±0.2	0.8±0.1*	0.8±0.2	0.6±0.1**
Lymphocytes, %	21.4±1.1	31.2±1.1	38.1±1.9*	31.2±1.1	22.5±1.4*/ **
Monocytes, %	3.6±0.5	4.0±0.5	4.1±0.6*	4.0±0.5	3.7±0.5*/ **
Basophiles, %	0.2±0.1	0.4±0.2	0.4±0.1*	0.4±0.2	0.3±0.1*/ **

Note. $p < 0.05$; *significant at comparison with control; ** at comparison of identical indexes in patients of group 1 and group 2.

Indicated hemogram changes could be manifestations of immune inflammatory processes, and also serve as the markers of endogenous

intoxication. Under the influence of conducted treatment in the patients of group 1 there was observed a tendency to decrease the signs of inflammation by day 7 of the hospital. In patients of group 2, receiving conventional therapy in combination with fullerene, the signs of inflammatory process in clinical blood analysis were already absent by day 5 of the treatment. It is necessary to note, that positive dynamics of hemogram indexes in the patients of group 2 was due to expressed immunotropic effect of fullerene, it also demonstrated absence of toxic influence on the system of blood formation.

Thus, additional indication of BAS “water solution of hydrated C₆₀ fullerene” to conventional therapy in patients with CHMA of toxic genesis had positive influence on the indexes of clinical blood analysis, which can be explained by anti-inflammatory and immunotropic influence of this medicinal agent.

2.3. The state of protein, enzymatic and pigmentary exchange in chronic hepatitis patients at combination of conventional therapy with fullerene

In the group of CHMA patients as for biochemical indexes there was also observed a positive dynamics in both subgroups of patients. However, these changes were more expressed in the subgroup of patients, treated with the proposed method. The indexes of protein exchange in the patients changed significantly, which manifested by decreased albumen content and increased globulins in serum before treatment.

Observed changes can indicate the presence of the syndrome of immune inflammation and hepatocellular insufficiency.

Thymol test indexes, indicating changes of colloid content of serum proteins and observed at mesenchymal inflammatory syndrome, were significantly increased.

In CHMA patients, at normal content of total serum bilirubin, there was observed significant increase of conjugated fraction and constituted 55.9% of total bilirubin. Obtained results indicate the presence of cholestasis syndrome of different degree of manifestation, which depended on the activity of pathological process in the liver.

Analyzing enzymatic exchange there was studied the content of serum indicative enzymes: AST (cytoplasmic mitochondrial), ALT (cytoplasmic); excretory enzymes: GGT and ALP.

Analyzing enzymatic exchange in CH patients there was estimated significant increase of activity of organ-specific indicator, as well as excretory enzymes, which was in direct dependency on the character of clinical manifestations of cytolysis and cholestasis syndromes. Thus, in the patients with CH of toxic genesis there were noted disturbances of protein, pigmentary and enzymatic exchange (Tabl. 2.2).

Table 2.2

The state of protein, pigmentary and enzymatic exchange in CHMA patients in the dynamics of treatment (M±m)

Indexes	Control n=20	Group 1		Group 2	
		Before treatment n=20	After treatment with conventional method n=20	Before treatment n=20	After treatment with proposed method n=20
Whole protein (g/l)	68.70±1.20	75.44±1.62	84.54±1.44	75.78±1.42	83.07±1.87*/*
Albumens (%)	56.55±0.98	52.25±1.92	54.97±2.12*	52.35±2.03	55.14±2.31*/*
Globulins (%)	7.69±1.14	6.65±0.71	6.96±0.69	6.71±0.79	7.96±0.93*/**
Alpha 1					
Alpha 2	8.8±0.38	9.82±0.47	9.78±0.42	9.81±0.46	9.51±0.36
Beta	11.09±0.58	12.9±0.51	11.43±0.36*	12.54±0.49	11.40±0.35*
Gamma	14.61±0.44	18.32±0.32	16.83±0.52*	18.52±0.54	16.45±0.41*
Thymol test (U)	2.04±0.11	3.37±0.15	2.89±0.13	3.38±0.16	2.26±0.11*
Total bilirubin (µmol/l)	11.76±0.64	17.53±0.66	13.71±0.63*	17.36±0.65	12.36±0.52*
Conjugated bilirubin (µmol/l)	4.5±0.19	9.72±0.27	7.87±0.14*	9.68±0.28	7.21±0.13*
AST (mmol.h./l)	0.43±0.02	0.75±0.21	0.62±0.10	0.78±0.13	0.43±0.09*
ALT (mmol.h./l)	0.38±0.07	0.56±0.11	0.43±0.08	0.59±0.13	0.39±0.06
GGT (mmol.h./l)	1.75±0.19	3.77±0.22	2.1±0.11*	3.63±0.21	2.05±0.09*
ALP (U)	0.65±0.15	2.25±0.43	1.55±0.38	2.17±0.41	1.58±0.39

Note: *- significance between identical indexes in each group, ($p < 0.05$),
 ** - significant between identical indexes after treatment between groups, ($p < 0.05$)

Analyzing protein, pigmentary and enzymatic exchange in CHMA patients, receiving conventional therapy, there was observed significant increase of albumens, decreases of gamma- and beta- globulins, total bilirubin and GGT.

In the subgroup of CHMA patients, receiving therapy with the proposed method, there were significantly increased alpha-1-globulins and significantly

decreased whole protein, albumens, beta-globulins, thymol test index, total bilirubin, conjugated bilirubin, AST and GGT, which supports potentiative action of fullerene on the restoration of the functional state of hepatocytes. Increase of whole protein level and normalization of fraction content in the patients of group 2 were also conditioned by protection against proteolytic “attack” against the background of the inflammatory process.

2.4. The state of lipid peroxidation and antioxidative protection system in patients with chronic hepatitis of toxic genesis at combination of conventional therapy with fullerene

Analyzing lipid peroxidation and indexes of antioxidative protection in the patients with CH of toxic genesis there was estimated activation of LPO processes and decrease of AOS (Tabl. 2.3).

At the same time, in CHMA patients there was noted significant increase of MDA content in serum and erythrocyte membranes, by 2.1 and 7 times, correspondingly. The levels of peroxidase, ceruloplasmin, SOD and SH-groups were significantly decreased.

Comparing clinical and biochemical indexes with the activity of antioxidative activity enzymes it is possible to assume, that functional state of the enzymes of antioxidative protection sufficiently reflects the peculiarities of the development of pathological process in the patients with CH of toxic genesis.

Probably, the patients with chronic hepatitis of toxic genesis have decreased rate of enzymatic utilization of superoxide radicals in plasma. Concomitantly they have increased ceruloplasmin secretion from the liver, which probably has compensatory significance, as ceruloplasmin protects lipid-containing macromolecules and lipoproteids against oxidative influence of oxygen radicals, acting as rapid “circulating” antioxidant.

After conducted therapy in the patients of both CHMA subgroups (Tabl. 3.2) there was observed restoration of the indexes of lipid peroxidation. At the same time, in the patients of the main subgroup, treated with the proposed method, MDA normalization in serum and erythrocytes, peroxidase and catalase, SOD and SH-groups occurred more rapidly and significantly.

Table 2.3

LPO and AOS indexes in CHMA patients in the dynamics of treatment (M±m)

Indexes	Control n=20	Group 1		Group 2	
		Before treatment n=20	After treatment with conventional method n=20	Before treatment n=20	After treatment with proposed method n=20
MDA in the serum ($\mu\text{mol/l}$)	0.62±0.11	1.28±0.14	1.11±0.09	1.25±0.13	0.58±0.05
MDA in erythrocytes ($\mu\text{mol/l}$)	7.48±0.63	12.53±0.76	10.52±0.54*	12.49±0.75	7.53 ±0.32*/**
Peroxidase (mmol ×min/l)	280.35±2.43	210.57±2.21	225.66±2.36*	210.47±2.2	275.44 ±2.52*/**
Catalase (mg)	16.36±0.19	14.74±0.14	15.88±0.26*	14.69±0.12	16.58 ±0.32*/**
Ceruloplasmin ($\mu\text{mol/l}$)	1.86±0.13	3.23±0.23	2.83±0.18	3.28±0.25	2.14 ±0.11*/**
SOD (u/ml)	13.53±0.53	12.96±0.20	13.08±0.23	12.99±0.21	13.46±0.3 2
SH-groups ($\mu\text{mol/l}$): - total	2.24±0.18	1.73±0.14	2.03±0.19	1.75±0.15	2.86 ±0.23*
- Nonprotein	0.75±0.13	0.65±0.08	0.68±0.09	0.66±0.08	0.86 ±0.12*/**
- Protein	1.49±0.17	1.08±0.07	1.38±0.12	1.06±0.06	1.65±0.23

Note: *- significance between identical indexes in each group, ($p < 0.05$),

** - significant between identical indexes after treatment between groups ($p < 0.05$).

In the patients of group 1 there was observed significant decrease of MDA in erythrocytes and increase of peroxidase and catalase, mainly due to protein ones, then in the patients, treated with a conventional method, which proves more expressed antioxidative action of the proposed scheme of treatment in comparison with conventional therapy. Fullerene manifested long-term antioxidative activity due to regulation of the level of free radicals in the focus of inflammation. This

peculiarity differs fullerene from other known antioxidants. Under the influence of BAS “water solution of hydrated C₆₀ fullerene” the patients with CH of toxic genesis had tendency to recovery of the secondary product of cell membrane peroxidation – MDA, activity of endogenous antioxidative system, proved by normalization of its key enzyme – SOD.

Use of fullerene substantially determines the intensity of lipid peroxidation of cell membranes. It is proved, that it can stabilize the membranes via stimulating the activity of glutathione peroxidase zinc-copper dependent superoxide dismutase. On the cell level fullerene activates polysome formation, inhibits iron-catalyzed free radical oxidation [5].

2.5. The state of lipid exchange in chronic hepatitis patients at combination of conventional therapy with fullerene

The indexes of lipid exchange in the patients with CHMA of toxic genesis had tendency to decrease or normalization in both groups of patients, treated with conventional and proposed therapies, but in group 2 the improvement occurred earlier and was more expressed (Tabl. 2.4).

Table 2.4.

The state of lipid exchange in CHMA patients in the dynamics of treatment (M± m)

Indexes	Control n=20	Group 1		Group 2	
		Before treatment n=20	After treatment with conventional method n=20	Before treatment n=20	After treatment with proposed method n=20
Total lipids (g/l)	5.81±0.16	8.37±0.19	6.92±0.14*	8.41±0.18	5.86±0.17*/**
Total phospholipids (g/l)	3.06±0.06	5.13±0.15	3.76±0.21*	5.17±0.21	3.27±0.18*/**
Total cholesterol (mmol/l)	4.77±0.09	7.83±0.21	5.31±0.19*	7.79±0.19	4.83±0.23*/**
Free cholesterol	0.46±0.00 7	2.32±0.43	1.29±0.39	2.38±0.41	0.96±0.38*/**
Conjugated cholesterol (mmol/l)	4.37±0.12	5.92±0.16	4.68±0.42	5.89±0.15	4.29±0.34
Triglycerides (U)	11.3±0.7	15.91±0.8 7	12.75±0.38*	15.89±0.91	11.74±0.42*/ *

Note: *- significance between identical indexes in each group, (p< 0.05),

** - significant between identical indexes after treatment between groups (p< 0.05).

Fullerene also has antioxidation and deintoxication properties [5].

Chronic hepatitis is a consequence of a great number of reasons, which determines polymorphism of clinical manifestations and different expressiveness

of the process activity in hepatic tissue. In recent years, there have been studied new pathogenetic mechanisms, leading to the development of chronic hepatitis. This was the ground for developing of new methods of treatment. However, the number of chronic hepatitis cases remains invariably high [3, 6]. Its frequency in the countries with transition economy has no tendency to decrease, which is connected with growth of chronic viral infections and intoxications, unfavourable ecological and social conditions of living. It requires systematization of the existing knowledge about pathogenesis, as well as development of new differentiative approaches to diagnostics and treatment of this pathology [9, 13, 24].

The results of the study allowed to draw the following conclusions: clinic of the disease typical for CH is manifested not only by expressed symptomatology, but also by presence of biochemical syndromes of cytolysis, cholestasis, immune inflammation and hepatocellular insufficiency with disturbance of immune reactivity, activation of LPO processes and decreased level of antioxidative protection.

It is possible to conclude, that the treatment of patients with chronic hepatitis should be differentiative with the use of corrective therapy, aimed at restoration of the function state of liver, metabolic processes in the organism.

The use of complex therapy in the patients with chronic hepatitis of toxic genesis with use of BAS “water solution of hydrated C₆₀ fullerene” allowed to obtain improved functional activity of the liver, normalization of clinical and biochemical serum indexes in comparison with conventional therapy, and also characterized by better tolerance. The use of fullerene in combination with Essentiale-N has positive influence on improvement of hepatocyte function, restoration of protein, lipid and enzymatic exchanges and, to a less degree, on pigmentary exchange, improves metabolic and oxidation-reduction processes in the organism, decreases inflammatory and fibrosing processes in the liver.

It should be noted, that conducted remedial measures stabilize pathological process in the liver, but do not lead to full recovery of the patient, so it is

reasonably for the patients to continue intake of BAS “water solution of hydrated C_{60} fullerene” and undergo regular medical check-up.

CONCLUSIONS

1. Use of BAS “water solution of hydrated C₆₀ fullerene” in complex therapy in the patients with chronic hepatitis has positive influence on the clinical course of the disease, functional state of hepatocytes and restoration of clinical and biochemical indexes. Fullerene determines the intensity of lipid peroxidation of cell membranes, stabilizes membranes, inhibits free radical oxidation, regulates metabolism, and enhances the intensity of restorative processes in the liver.

2. Use of fullerene in combination with complex therapy in case of chronic hepatitis of toxic genesis promotes normalization of indexes of clinical blood analysis in the patients with chronic hepatitis of toxic genesis, which was associated with expressed anti-inflammatory effect of the treatment and absence of toxic influence.

3. Normalization of indexes of protein exchange in the patients with chronic hepatitis of toxic genesis against the background of complex therapy in combination with BAS “water solution of hydrated C₆₀ fullerene” was conditioned by improvement of functional state of hepatocytes, inhibition of proteolytic “attack”.

4. Fullerene in combination with conventional therapy has significant antioxidative abilities, associated with decrease of free radical processes.

5. Inclusion of BAS “water solution of hydrated C₆₀ fullerene” into complex therapy of the patients with chronic hepatitis of toxic genesis promotes the improvement of general state of the patients, significantly decrease clinical manifestations of the disease, rate of the cytolysis syndromes, immune inflammation, and hepatocellular insufficiency.

6. Use of BAS “water solution of hydrated C₆₀ fullerene” in the patients with chronic hepatitis in a complex with hepatoprotectors in comparison with conventional therapy allows to achieve more expressed improvement of functional activity of hepatocytes, restoration of adaptive responses of the organism, indexes of metabolic processes in the organism.

PRACTICAL RECOMMENDATIONS

Use of biologically active supplement “water solution of hydrated C₆₀ fullerene” can be recommended in a complex therapy of the patients with chronic hepatitis of toxic genesis according to the following regimen: 50 ml 3 times a day 15-20 min before meals for the first 3 days, then 50 ml twice a day 15-20 min before meals for 3 days, and further once a day 15-20 min before meals for 15 days.

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