USE OF INFORMATION PARAMETERS AS CRITERION FOR DETERMINATION OF BIOLOGICAL ACTIVITY OF TISSUE PREPARATIONS

David A. Areshidze[1]*, Lyudmila D. Timchenko[2], Igor V. Rzhepakovsky[2], Maria A. Kozlova[1], Ivan A. Syomin[1].

[1]Researching Laboratory of Experimental Biology and Biotechnology, Moscow State Regional University, 10A Radio st., Moscow, RUSSIAN FEDERATION.

[2]Laboratory of experimental immunomorphology, immunopathology and immunobiotechnology, North Caucasus Federal University, 1 Pushkin st., Stavropol, RUSSIAN FEDERATION.

notbio@mgou.ru.

*Abstract:*Possibility of use of the information parameters characterizing adaptational and regenerative opportunities of organ as potential criteria of an assessment of biological activity of tissue preparations is investigated in this research. Influence of tissue preparation «STEMB» on a morphofunctional condition of a liver of rats at experimental toxic damage of organ was studied. The assessment of a morphofunctional condition of a liver under the influence of a preparation was carried out as with use of traditional morphological, biochemical and histologic parameters, and by means of information parameters. As a result of research it is established that hepatoprotective activity of preparation «STEMB» is confirmed both with results of traditional techniques of an assessment, and with informational parameters, that allows to draw a conclusion on possibility of use of information parameters as criterion of an assessment of biological activity of tissue preparations.

Key words: liver, tissue preparation, biological activity, entropy

1. INTRODUCTION

Now the increasing amount of researches is devoted to new groups of tissue preparations because of their potential biological activity. It may be explained by the fact that the wide range of biological effect of the substances containing in the remedies made of tissues of animals and plants provides them applicability in various branches of biology, medicine and veterinary[1-5].

Development of modern biotechnological and pharmaceutical technologies allow to improve process of receiving of biologically active agents from natural raw materials, to specify the active components of preparations and mechanisms and targets of their action [6-7]. Biologically active remedies are made from tissues of a wide range of species of eukaryotes. Biological activity is peculiar for extractions from organisms of invertebrates and vertebrates, and also from vegetative and generative organs of plants [8-11].The whole organisms, or their immunocompetent organs, skin, liver, muscles, placenta and chorion, blood serum and whole blood, antlers, fragments of tissue of an embryo of various species or whole embryo completely can be feedstock for receiving biologically active agents [12-14]. Embryonic tissues are one of the most promising sources for receiving the biostimulating preparations, and the most convenient for use for this purpose is the chicken embryo [15-18].

Effect of bioactive preparations of natural origin is caused by the contents in them of the natural composites similar with constructs of living cell or tissue. These composites carry out stimulation in process of functional inquiry of an organism. They may serve as a subsidiary source of the major nutrients, bioactive peptides, growth factors, and also the vitamins and mineral nutrition for an organism. Natural bioactive preparations contain so called biogenous stimulators which have impact on the main parties of a metabolism. This impact is expressed in changes of metabolic conversion and energy

processes of an organism [19-21]. Biogenous stimulators and adaptogens are one of bases of efficiency of preparations of natural origin[22-24].These concepts are meant as group of biologically active agents of non-uniform chemical structure; it is usual to carry to them the low-molecular organic compounds containing in living cells. In particular, this role belongs to organic acids (dicarboxylic and tricarboxylic acids, RNA, DNA etc.), humin substances, phospholipids, vitamins, microelements and low molecular weight peptides [23].

Experiments show that remedies made of living tissues show considerable effectiveness at application at smoldering pathological processes like inflammation, degeneration, atrophy, tumorogenesis, etc., as they activates immune and regenerative functions of an organism. The natural pharmacology products are effective at eye and skin diseases, diseases of musculoskeletal system, in treatment of burn injuries, at oncological practice, at treatment of endocrinopathiesand Alzheimer's disease [24-27].Tissue preparations also show the properties making them applicable as adaptogens at high physical activities at sportsmen and manual workers and also atpersons having strong psychoemotional loadings [28-31].

Derivatives of living tissues are widely applied also in veterinary science where their application is expedient because of necessity of large volumes and high efficiency of the using substances and also their potential safety for the end user of food and other products of farm animal origin [32-35].

In this regard, the matter of criteria of determination of efficiency of action of tissue biological preparations is important now.

For determination of efficiency of use of biologically active substances and biological products (which are in essence the highly active catalyst of biological processes, including their ability for essential change and violation in the existing tissue homeostasis through changing of both adaptive and regenerative specifics of tissue), very wide range of methods and techniques is now applied.

Traditionally, tests of stimulators are carried out on laboratory animals [36]. Actually, speaking about influence of this or that biostimulator on the studied organ or system, we estimate the level of adaptational and regenerative opportunities of the studied system, but the uniform integrative criteria of an assessment of these opportunities are not present.

At all wide range of approaches to a question of determination of level of adaptational and regenerative processes in norm, at pathologies, as a result of biostimulation[37-50], it isn't possible to mark out an adequate criteria of an assessment of intensity of these processes which wouldn't demand confirmation by any other methods and reflect unity of a structure and functions of the studied systems.

One of the methods applied to an assessment of adaptational and regenerative processes is use of information parameters. In particular, this method finds application in medicine. So, entropy was used for definition of violations of cardiac activity at fetuses at the last stages of prenatal ontogenesis [51]. Also entropy was applied to the description of regularities of secretion of hormones[52-53]. Very widely information parameters are used at research of cardiovascular system [54-55], in neurophysiology [56-57], and also in various areas of science studying cellular, tissue and organ structures [58-65].

Research of possibility of an assessment of information parameters as the criterion displaying an effect of application of a tissue biostimulator seemed to us actual. For this purposethe assessment of influence of the tissue preparation «STEMB» on a morphofunctional condition of a liver of rats at experimental toxic hepatitis was carried out by us. Thus the assessment was carried out with use traditional morphological, physiological, histochemical and histologic techniques, and also by an assessment of information condition of organ. The choice of the preparation «STEMB»is caused by that its biostimulation influence on a liver is shown by earlier conducted researches. Besides, it is shown that the information parameters applied by us reflect the level of adaptational and regenerative resources of organ[66].

2. MATHERIALS AND METHODS

2.1.Animals

Male Wistar Albino rats of body weights ranging from 170 g to 200 g were used in the study. The animals were fed with standard pellet diet and water ad libitum. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (30±2ºC). All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

2.2.Treatment Design

150 animals (Male Wistar Albino rats) were randomized and divided into three groups on fifty animals in each group. One of groups served as intact control. Animals in Group Iwere

inhaled by carbon tetrachloride to 2 min. per day for 6 days (control group). Rats in Group II were inhaled carbon tetrachloride to 2 min a day for 6 days, but at the same time received hypodermic injections of «STEMB» in a dosage of 3 mg/kg.bw at 1-5th days of research. Selection of carbon tetrachloride (CCl_4) as an agent acting on the liver is caused by the fact that this substance is a direct liver poison, widely used in experimental medicine and biology. Selecting of the liver-toxic and exposure method is determined by the fact that the use of carbon tetrachloride under this scheme provides the appearance and development of reversible changes in liver at tissue and organ level.On the7th day of research animals were sacrificed in carbon dioxide chamber.

2.3.Weight Measurements

All rats were weighed in grams. Weighing was made at the beginning and at the end of research in each group. At the end of experiment it was measured absolute (in grams) and relative mass of a liver of rats.

2.4.Biochemical Analyses

Levels of uric acid, creatinine, total bilirubin, of an alanineaminotransferase (ALT) and aspartataminotransferase (AST) in blood serum were investigated by means of the biochemical StatFax3300 analyzer (USA) by means of sets of Spinreact firm (Spain).

2.5.Histopathological Analysis

Small portions of liver were taken and fixed in 10% formaldehyde. After several treatments for dehydration in alcohol, sections having 5μm thickness were cut. Sections were subjected to stain with hematoxylin and eosin, and then the histopathological analysis was carried, including determination of mitotic, apoptotic and necrotic indexes in liver. At hematoxylin and eosin stained sections we determined quantity of mitotic and necrotic cells. At sections stained by methylene blue-azure II with afterstain by fuchsine we determined a quantity of apoptotic cells. Visualization was performed using a microscope Nikon Eclipse 80i at 900x magnification. All studies were made for 10 fields of view on each section.

Apoptotic index was calculated by the formula:

$$AI=N_a/N\times100\%,$$

where N_a - the number of apoptotic cells; N - total number of cells in the test population.

Mitotic index was calculated by the formula:

$$MI=N_m/N\times100\%,$$

where N_m - number of mitotic cells; N - total number of cells in the test population.

Necrotizing index was calculated by the formula:

$$NI=N_n/N\times100\%,$$

where N_n- number of necrotic cells; N - total number of cells in the test population.

2.6.Morphometric Studies

All measurements were taken with useof image analyzer "Videotest" at hematoxylin and eosin stained sections. Study was made for 10 fields of view on each section.

2.7.Studies ofthe Information Condition of the Systemof the Organs

To determine the information status at focal lesions of the liver, pieces of tissue were taken from the least altered areas on the border of macroscopically distincted lesions. In case of visual homogenity of organ material for research was taken from any part of it.

Based on the concept of information in a tissue system as the displaying of the diversity of morphology and function of the process for assessing the information status of organs and tissues have been proposed and tested the such indicators - information morphological capacity (H_{max}), information morphological entropy (H), information morphological organization (S), the relative morphological entropy (h) and redundancy (R) [67-70]. The baseline characteristics, which are used to calculate these parameters, can vary widely (the linear dimensions of the structures, their quantity, etc.). In our study we defined the volume of the nuclei of hepatocytes.

Information morphological capacity H_{max}, which means the maximum structural diversity, was calculated by formula:

$$H_{max}=\log_2 n,$$

where n - number of classes.

Next, we made the calculation of the real structural diversity H. Real structural diversity is the parameter that clearly illustrates the degree of determinism of morphofunctional system in time and space [67-70].The calculation was made using the formula:

$$H=-\Sigma P_i \log_2 P_i,$$

whereΣP_i is the sum of probabilities of staying of the measured parameter of cells in a one of existing classes; $\log_2 P_i$ - logarithm of the probability of staying in one of the possible classes. In this case, the value of $P_i$is defined as the classical probability.

Knowing the maximum and actual structural diversity, we can calculate the organization of the system (S), the difference between the max-

imum possible and the real structural diversity (implemented structural diversity). This parameter, in our opinion, displays the state of the system adaptability to date. To determine the value of this parameter we used the formula:

$$S=H_{max}-H.$$

It is necessary to consider that when H = H_{max}, the system is deterministic, but such relation to the vast majority of permissible is possible only in theory.

Then we determined the coefficient of relative entropy of the system (or the coefficient of compression of information) h by formula:

$$h=H/H_{max}.$$

High levels of relative morphological entropy provide evidence of the disorder of the system and of significantly reduction of its structural integrity.

The coefficient of the relative organization of the system (redundancy factor) R is given by formula:

$$R=(S/H_{max})\times 100\%.$$

With these data, the researcher have the opportunity to calculate the equivocation of the system(the value of reliability) ***e***:

$$\boldsymbol{e}=(H_p-H_n),$$

where H_n - real structural diversity in normal, H_p - real structural diversity in pathology.

2.8.Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 packed program. Data were presented as mean ± standard deviation unless noted as different. Difference between the control and experimental groups was analyzed using Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Effect on body and liver mass

By results of research it is established that at experimental toxic hepatitis (I group) there is a decrease in body weight of animals, and also in absolute and relative mass of a liver. At the same time, similar parameters of animals of the second experimental group are reliable higher, than in the first, and don't differ significantly from control parameters. (Table 1).

Table 1. Some morphological parameters of a liver of rats at an assessment of biological activity of the tissue preparation «STEMB».

Group	Body weight, g	Absolute mass of liver, g	Relativemassofliver, %
Control group (n=50)	209.30±5.61	7.60±0.37	3.62±0.30
I group (n=50)	181.40±9.14*	9.10±0.44*	5.02±0.37*
II group (n=50)	201.80±12.51▲	7.69±0.91▲	3.81±0.31▲

Hereinafter: control group – intact animals; I group – the animals inhalated by CCl_4; II group – the animals inhalated by CCl_4 and at the same time treated with tissue preparation «STEMB». * P≤0,0 5 – in comparison with control group, ▲P≤0,05 – the II group in comparison with the I group.

3.2.Effect on blood serum

Results of biochemical researches testify that at experimental toxic hepatitis there is an essential deviation of all studied parameters of blood serum, except for the total protein, in comparison with norm.

In the second group, there is insignificant change of the studied parameters in comparison with control, except for the level of AST, but also permanent is a level of the total protein (Table 2).

Table 2. Some biochemical parameters of blood serum ofratsat an assessment of biological activity of the tissue preparation «STEMB».

Group	AST, u/l	ALT, u/l	Total bilirubin, g/l	Total protein, g/l	Glucose, μmol/l
Control group (n=50)	114.2±2.78	179.5±3.72	9.82±0.38	75.24±5.23	6.58±0.52
I group (n=50)	168.8±22.14*	303.1±33.74*	23.88±1.42*	76.52±4.48	11.84±1.1*
II group (n=50)	148.51±25.98*	190.11±37.18▲	10.74±1.40▲	74.59±4.16	7.61±1.14▲

3.3. Effect on mitotic, apoptic and necrotic activity

In a liver of animals of group II there is an essential decrease in mitotic and apoptotic activity at simultaneous increase in number of necroses. Under the influence of «STEMB» the quantity of cells in a condition of a mitosis and a necrosis reaches control level, but thus the level of apoptotic activity is higher, than in a liver of intact rats and animals of the second group (Table 3).

Table 3. Mitotic, apoptoticand necrotic indexesof rat liverat an assessment of biological activity of the tissue preparation «STEMB».

Group	Mitotic index,%	Apoptotic index,%	Necrotic index,%
Control group (n=50)	6.41±0.37	1.87±0.22	0.70±0.09
I group (n=50)	2.41±0.36*	0.71±0.20*	14.14±2.56*
II group (n=50)	6.91±0.48▲	3.12±0.30*▲	1.21±0.25▲

3.4.Histopathologic findings

The histopathologic feature of a liver of intact rats complied with norm (Fig. 1,2).

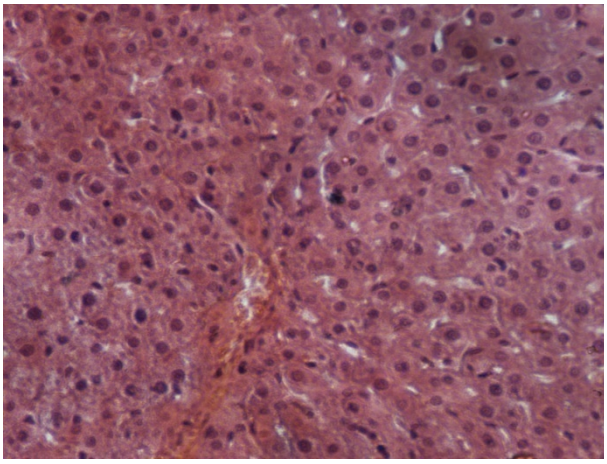

Figure 1. Liver of intact rat.
×400, H&E

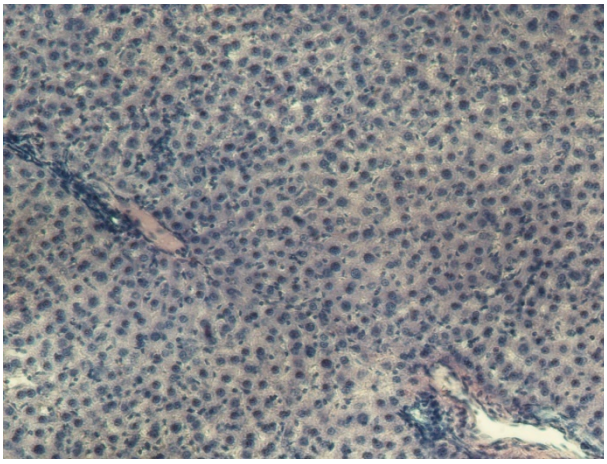

Figure 2. Liver of intact rat.
×200, H&E

At the pathomorphologic examination of the liver of rats exposed to carbon tetrachloride was found that the organs had a red color, sometimes with yellow or gray tint. About 20% of rat liver were spotty. The organs were loose and easily tearing, with blood oozing on the cut. The histological study revealed a pronounced attenuation of hepatic beams. Hepatocytes were swollen, with turbid cytoplasm; the edges of the cells were not clearly discernible; the nuclei were also swollen, bright, with blurred edges. In hepatocytes were distinctly observed clear vacuoles. Detached hepatocytes were in state of granular dystrophy. The vessels of the liver in different parts of the cut were irregularly expanded and filled with blood, in the field of triads were observed the signs of mild perivascular mesenchymal reaction.

In a number of cases the layers of connective tissue, thickened and infiltrated by small cells, are observed, primarily in the fields of triads. Blood vessels (central vein capillaries) of the liver are dilated (hyperemia of blood vessels), the permeability of the walls of vessels for the blood cells is increased, the focal hemorrhages are observed. Among the cells is observed a large number of white blood cells, the macrophages. Some single cells are very large and are in fact a continuous vacuole.

In 80% of cases multiple foci of necrosis of different sizes are observed, in which the structural elements of the individual cells are not rendered, and the tissue of liver is a homogeneous structureless mass. In 47% of cases the extensive necroses are noted. The observed changes indicate the development of typical toxic damage of liver at animal groups. However, at some rats were established characteristic of the microfocal alterative inflammation. A significant part of animals had typical signs of acute toxic hepatitis with high intensity of tissue damage (alterant hepatitis). Some animals had severe hepatic steatosis with necrotic component (Fig. 3,4).

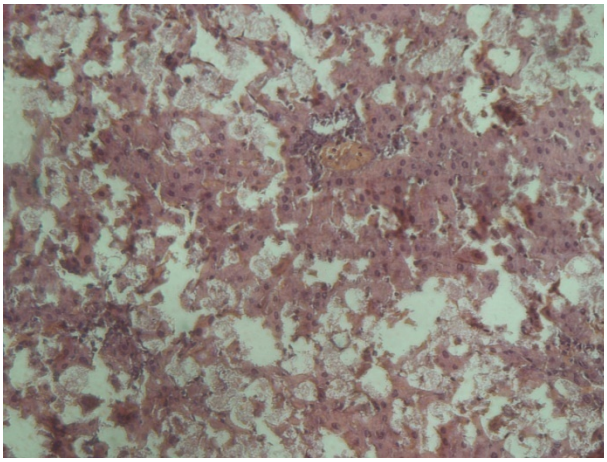

Figure 3. Liver of rat from group I.
×200, H&E.

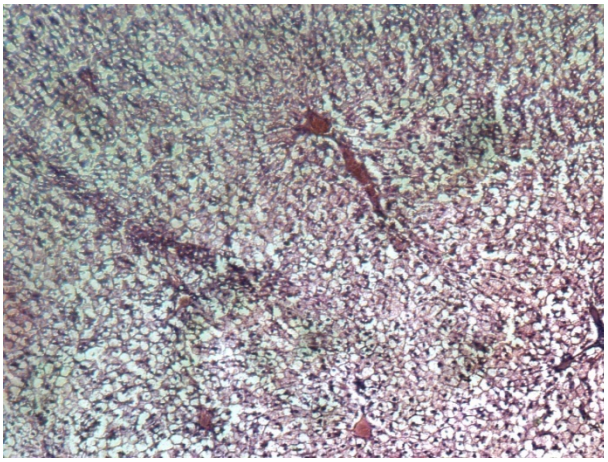

Figure 4. Liver of rat from group I.
×100, H&E.

At inhalation by CCl4 with the simultaneous use of the «STEMB», pathological changes in the liver are the much less severe (Fig.2). In particular, hepatic beams and lobular structure in the liver of all animals stay intact. A few fields of malnutrition alternate with areas represented with binuclear and intact hepatocytes (signs of recovery) or hepatocytes in a condition of the initial stage of granular dystrophy, fatty degeneration occurs in 21%.

Also, there are substantially less hepatocytes in a state of necrosis. It is noted the absence of focal hemorrhages, capillaries are moderately bloodshot, and there are no signs of swelling. The permeability of vessels is also considerably less. Vessels in the triads are moderately dilated. Over 29% of hepatocytes have small vacuoles (Fig. 5,6).

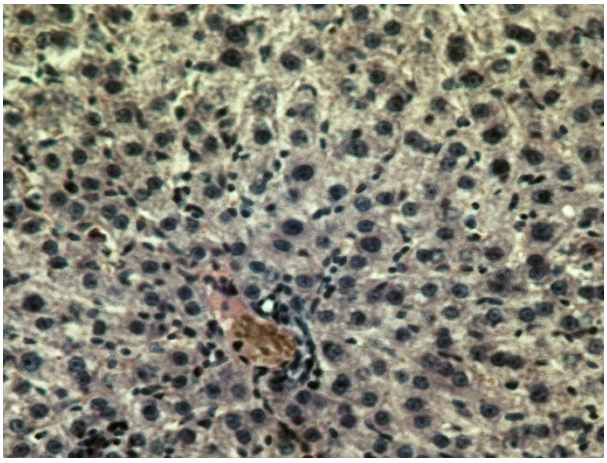

Figure 5. Liver of rat from group II.
×400, H&E.

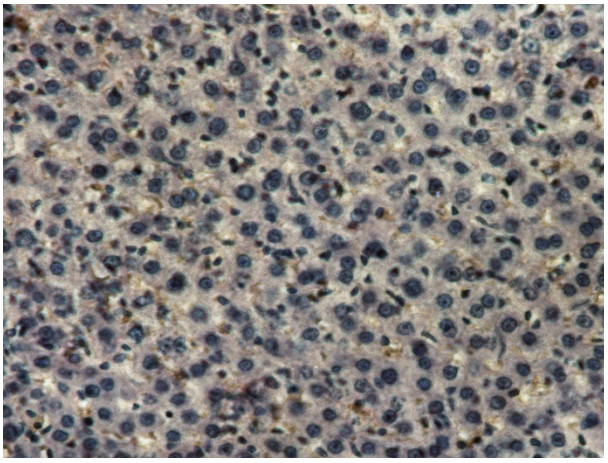

Figure 6. Liver of rat from group II.
×400, H&E.

3.5. Effect on Informational condition

At comparing with control in a liver of both studied groups is observed an increase in level of parameters H and h, decrease of level of parameters S and R. At the same time, changes of the studied information parameters in the group II are authentically differ than parameters of the first group I (Table 4).

Table 4. Informational parameters of liver of ratsat an assessment of biological activity of the tissue preparation «STEMB».

Group	H_{max}(bit)	H (bit)	S (bit)
Control group (n=50)	3.32±0.002	2.518±0.023	0.8018±0.023
I group (n=50)	3.32±0.002	2.719±0.024*	0.6013±0.024*
II group (n=50)	3.32±0.002	2.569±0.029*▲	0.7508±0.029*▲

	h (bit)	R (%)	*e* (bit)
Control group (n=50)	0.7585±0.007	24.15±0.71	-
I group (n=50)	0.8189±0.007*	18.11±0.73*	0.201±0.010
II group (n=50)	0.7739±0.009*▲	22.61±0.89▲	0.051±0.006▲

4. DISCUSSIONANDCONCLUSION

The conducted research shows that tissue preparation «STEMB» has hepatoprotective effect at experimental toxic hepatitis at rats. It is shown in the expressed normalization of the majority of the studied biochemical parameters, preservation of morphological integrity of a liver, maintenance of a tissue homeostasis of organ. It is remarkable that the leading mechanism of cellular death at toxic hepatitis and simultaneous application of the preparation «STEMB» is not the necrosis, but apoptosis which intensity increases in a liver of animals of the second experimental group even in comparison with control.

By results of the analysis of information condition we noted an increase in parameter H in both groups testifies thatthere is an increase in degree of disorder of tissue system of organ. Also in both groups the level of structural integrity of system goes down, that is confirmed by increase in value of pereameter h. At the same time decrease in the values of parameters S and R speaks about decrease of adaptational opportunities of organ and about decrease in quantity of the structural elements capable to provide the necessary level of adaptation and the necessary level of structural and functional integrity of organ.

At comparing of values of the studied parameters, the intergroup distinctions are clearly evident.

Values of parameters H and h increased in group treated with the preparation to 2.02% and 2.03% (that is no more than for 5% of norm) in comparison with control, but in group without application of a preparation these parameters increased for 7.98% and 7.96% respectively. These data testify to success of biostimulation at using the preparation «STEMB». Besides, values of parameters S and R went down in the range up to 10% from norm, to 6.36% and 6.38% respectively, whereas in group without application of a preparation these parameters decreased to 25.0% and 25.01%. The increase of parameter ***e*** to level of 0.051 bits (which isn't exceeding 0.1 bits), is also revealed in group treated with «STEMB», whereas in group without application of tissue preparation it made up more than 0.2 bits.

Thus, decrease in morphological and functional integrity of a liver, as it is shown by results of the analysis of information condition of organ, is more expressed at animals of the first group. At the same time the studied information parameters characterizing a liver of rats of the second experimental group though speak about certain changes in structure and function of organ, as well as earlier described results of biochemical, morphological and histologic researches, confirm hepatoprotective effect of preparation «STEMB». These facts allow to claim that the information investigations used in research can be applied as the criteria characterizing biological activity of tissue preparations.

ACKNOWLEDGEMENTS

The study was conducted under Task number 2014/2016 on the implementation of public works in the field of scientific activities of the

base portion of the state task of the Ministry of Education and Science of the Russian Federation. Financial support of research was carried out by Moscow State Regional University and by the Ministry of Education and Science of the Russian Federation, within performance of a basic unit of the state task (2014/2016).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Sreemantula S, Nammi S, Kolanukonda R, Koppula S, Boini KM. Adaptogenic and nootropic activities of aqueous extract of Vitis vinifera (grape seed): an experimental study in rat model. BMC. Complement Altern Med,2005; 5(1): 1-8.
2. Alves RRN, Alves HN. The faunal drugstore: Animal-based remedies used in traditional medicines in Latin America. J Ethnobiol Ethnomed 2011; 7(9): 1-43.
3. Alves RRN, Lima HN, Tavares MC, Souto WMS, Barboza RRD, Vasconcellos A. Animal-based remedies as complementary medicines in Santa Cruz do Capibaribe, Brazil. BMC Complement Altern Med 2008; 8 (44): 1-9. BMC Complement Altern Med 2004; 5(1): 1-8.
4. Chakravorty J, Meyer-Rochow VB. Ghosh, S. Vertebrates used for medicinal purposes by members of the Nyishi and Galo tribes in Arunachal Pradesh (North-East India). J Ethnobiol Ethnomed 2011; 7 (13): 1-14.
5. Vallejo JR, Gonzales A. Fish-based remedies in Spanish ethnomedicine: a review from a historical perspective. J Ethnobiol Ethnomed 2014; 10(37): 1-31.
6. Yagi A. Bioactive constituents of a human placenta hydrolysate – from biogenic stimulants reported by Filatow to biological markers of collagen generation. Nat Med 2004; 58(4): 121-131.
7. Hosseini SV, Hamzeh A, Moslemi M, Lashkan AB, Iglesias A, Feas X. Effect of delayed icing on biogenic amines formation and bacterial contribution of iced common carp (Cyprinus carpio). Molecules 2013; 18: 15464-15473.
8. Cooper EL, Balamurugan M, Huang CY, Tsao CR, Heredia J., Tommaseo-Ponzetta M., Paoletti, M.G. Earthworms Dilong: ancient, inexpensive, noncontroversial models may help clarify approaches to integrated medicine emphasizing neuroimmune systems. Evid Based Complement Alternat Med 2012: 1-11.
9. Cooper EL, Hirabayashi K. Origin of innate immune responses: revelation of food and medicinal applications. J Tradit Complement Med 2013; 3(4): 204-212.
10. Vallejo JR, Gonzales A. The use of the head louse as a remedy for jaundice in Spanish folk medicine: an overview. J Ethnobiol Ethnomed 2013; 9 (52): 1-8.
11. Gonzales JA, Vallejo JR. The scorpion in Spanish folk medicine: a review of traditional remedies for stings and its use as a therapeutic resource. J Ethnopharmacol 2013; 146(1): 62-74.
12. Alves RRN, Rosa IL. Why study the use of animal products in traditional medicines? J Ethnobiol Ethnomed 2005; 1(5): 1-5.
13. Mobley CB, Toedebusch RG, Lockwood CM, Heese AJ, Zhu C, Krieger AE et al. Herbal adaptogens combined with protein fractions from bovine colostrum and hen egg yolk reduce liver TNF-α expression and protein carbonylation in Western diet feeding in rats. Nutr Metab 2014; 11(19): 1-12.
14. Oh TH, Markelson GJ. Dependence of in vitro myogenesis on a trophic protein present in chicken embryo extract. Proc Natl AcadSci 1980; 77 (11): 6922-6925.
15. Yadgary L, Wong EA, Uni Z. Temporal transcriptome analysis of the chicken embryo yolk sac. BMC Genomics 2014; 15(1): 690.
16. Alibardi L, Mlitz V, Eckhart L. Immunolocalization of scaffoldin, a trichohyalin-like protein, in the epidermis of the chicken embryo. Anat Rec 2015; 298(2): 279-287.
17. Chakraborty PD, Bhattacharyya D. Aqueous extract of human placenta as a therapeutic agent, In: Recent Advances in research on the human placenta. InTech, Rijeka 2012: 77-92.
18. . Choi S-W, Moon S-H, Yang HJ, Kwon DY, Son Y-J, Yu R, Kim A. et al. Antiresorptive activity of Bacillus-fermented antler extracts: inhibition of osteoclast differentiation. Evid Based Complement Alternat Med 2013: 1-9.
19. Chandika P, Ko S-C, Jung W-K. Marine-derived biological macromolecule-based biomaterials for wound healing and skin

tissue regeneration. Int J Biol Macromol 2015; 77: 24-35.
20. Gupta V, Gupta A, Saggu S, Divekar HM, Grover SK, Kumar R. Anti-stress and adaptogenic activity of L-Arginine supplementation. eCAM. 2005; 2(1): 93-97.
21. Panossian A, Wilkman G, Kaur P, Asea A. Adaptogens stimulate neuropeptide Y and Hsp72 expression and release in neuroglia cells. Front Neurosci 2012; 6(6): 1-12.
22. Pawar VS, Shivakumar H. A Current status of adaptogens: natural remedy to stress. Asian Pac J Trop Dis 2012; 2(1): 480.
23. Kashiwagi, S., Brauns, T., Gelfand, J., Poznansky, M.C. Laser vaccine adjuvants: History, progress, and potential. Hum Vaccin Immunother 2014; 10(7): 1892-1907.
24. Tolli H, Kujala S, Jamsa T, Jalovaara P. Reindeer bone extract can heal the critical-size rat femur defect. Int Orthop 2011; 35: 615-622.
25. Di Nicola A, Di Nicola,V. Self-repair in degenerative joint disease. Curr Aging Sci 2013; 5(3): 273-287.
26. Vengerovskii AI, Burkova VN, Iudina NV, Iatsenkov AI. Antiproliferative effect of polar lipids of maral antlers and peat in prostate benign hyperplasia model. Eksp Klin-Farmakol 2013; 76(5): 27-31.
27. Skopinski P, Balan BJ, Kocik J, Zdanowski R, Lewicki S, Niemcewicz M, Gawrychowski K et al. Inhibitory effect of herbal remedy PERVIVO and anti-inflammatory drug Sulindac on L-1 sarcoma tumor growth and tumor angiogenesis in Balb/c mice. Mediators Inflamm 2013: 1-10.
28. Yuan H-D, Kim JT, Kim SH, Chung SH. Ginseng and diabetes: the evidences from in vitro, animal and human studies. J Ginseng Res 2012; 36(1): 27-39.
29. Oliynyk S, Oh S. Actoprotective effect of ginseng: improving mental and physical performance. J Ginseng Res 2013; 37(2): 144-166.
30. Kumju Y, Eun-Young Y, Jinhyuk L, Ji-Young K, Jae-Sam H, Woo-Sik J. Oleic acid and linoleic acid from Tenebrio molitor larvae inhibit BACE1 activity in vitro: molecular docking studies. J Med Food 2014; 17(2): 284-289.
31. Jiang N, Zhang S, Zhu J, Gao X. Hypoglycemic, hypolipidemic and antioxidant effects of peptides from red deer antlers in streptozotocin-induced diabetic mice. Tohoku J Exp Med 2015; 236(1): 71-79.
32. Moreau M, Dupuis J, Bonneau NH, Lecuyer M. Clinical evaluation of a powder of quality elk velvet antler for the treatment of osteoarthrosis in dogs. Can Vet J 2004:45; 133-139.
33. Confessor MVA, Mendonca LET, Mourao JS, Alves RRN. Animals to heal animals: ethnoveterinary practices in semiarid region, Northeastern Brazil. J Ethnobiol Ethnomed 2009; 5 (37): 1-9.
34. Chen J, Woodbury MR, Alcorn J, Honaramooz A. Dietary supplementation of female rats with elk velvet antler improves physical and neurological development of offspring. Evid Based Complement Alternat Med 2012: 1-7.
35. Gisbert E, Skalli A, Fernandez I, Kotzamanis Y, Zambonino-Infante JL, Fabregat R. Protein hydrolysates from yeast and pig blood as alternative raw materials in microdiets for gilthead sea bream (Sparusaurata) larvae. Aquaculture 2013; 338-341: 96-104.
36. Churoasik S, Endelein, Bauer R, Grabner W. Evidence for antirheumatic effectivness of Stewed Herbautric aedioicae in acute arthritis: a pilot study. Phytomedicine1997; 4: 105-108.
37. Halboos MHN, Spectrophotometric Method for Determination and Biological Activity of Trace Amount of Cd(II) by bis(2-((pyridin-2-ylimino)methyl)phenyl)-4,4'-(diazene-1,2-diyl)dibenzoate. Int J Sci Res 03/2015; 4(3):2222-2226.
38. GuanS,·Peng C, Li H, XuS,·Yuan Z. Determination of biological activity of extract from hirudo by N-benzoyl-L-arginine ethyl ester. Zhongguo Zhong Yao Za Zhi 08/2010; 35(15):1990-2.
39. Torii Y, Goto Y, Takahashi M, Ishida S,·Harakawa T,·Sakamoto T et al. Quantitative determination of biological activity of botulinum toxins utilizing compound muscle action potentials (CMAP), and comparison of neuromuscular transmission blockage and muscle flaccidity among toxins. Toxicon 2009; 55(2-3):407-14.
40. Zhang H, Zhang X, Xu B. Analysis and determination of biological activity of short-chain peptides from porcine brain hydrolysate. J Pharm Biomed Anal 2005; 37(2):333-9.
41. Katafuchi T, Hamano K, Minamino N. Identification, structural determination, and biological activity of bovine and canine calcitonin receptor-stimulating peptides Biochem Biophys Res Commun 02/2004; 313(1):74-9.
42. Penkov D, Dimitrovs S, Andronova V, Milieva E, Murdjeva M, Stanimirova I et al.

Biological activity of bulgarian Folia Betulae dry extract. Int J Pharm PharmaceutSci 07/2015; 7(7): 124-136.
43. Luesch H, Yoshida WY, Moore RE, Paul VJ, Moorberry SL. Isolation, structure determination, and biological activity of Lyngbyabellin A from the marine cyanobacterium lyngbyamajuscula. J Nat Prod 06/2000; 63(5):611-5.
44. Chopin V, Matias I, Stefano GB, Salzet M. Amino acid sequence determination and biological activity of therin, a naturally occuring specific trypsin inhibitor from the leech Theromyzon tessulatum. Eur J Biochem 07/1998; 254(3):565-70.
45. Shulte M, Brecht-Krauss D, Heymer B, Guhlmann A, Hartwig E, Sarkar MR., Schulte M, Brecht-Krauss D, Heymer B, et al. Fluorodeoxyglucose positron emission tomography of soft tissue tumours: is a non-invasive determination of biological activity possible. Eur J Nucl Med 06/1999; 26(6):599-605.
46. Takei Y, Takano M, Itahara Y, Watanabe TX, Nakajima K, Conkin DJ et al. Rainbow Trout Ventricular Natriuretic Peptide: Isolation, Sequencing, and Determination of Biological Activity. Gen Comp Endocrinol 01/1995; 96(3):420-6.
47. Fumiere M, Fumiere M, Poltronieri H, Endringe R. Seasonality modifies rosemary's composition and biological activity. Ind Crop Prod 2015; 70: 106-118.
48. Moreta C, Tena M-T, Kannan K. Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals. Environ Res 08/2015; 142:452-460.
49. Preuett B, Leeder S, Abdel-Rahman S. Development and application of a high-throughput screening method to evaluate antifungal activity against trichophyton tonsurans. J Biomol Screen 07/2015; DOI:10.1177/1087057115594751.
50. Chakraborty M, Karmakar I, Haldar S, Nepal A, Haldar P. Anticancer and antioxidant activity of methanol extract of Hippophae Salicifolia in EAC induced swiss albino mice. Int J Pharm Pharmaceut Sci 06/2015; 7(8): 24-32.
51. Pincus SM, Keefe DL. Quantification of hormone pulsatility via approximate entropy algorithm. Am J Physiol 1992; 262: 741-754.
52. Pincus SM, Viscarello RR. Approximate entropy: a regularity measure for fetal heart rate analysis. Obstet Gynecol 1992; 79 (2): 249-255.
53. Veldhius JD, Roelfsema F, Iranmanesh A, Carroll B, Keenan D, Pincus S. Basal, Pulsatile, Entropic (Patterned), and Spiky (Staccato-like) Properties of ACTH Secretion: Impact of Age, Gender, and Body Mass Index. J Clin Endocrinol Metab 2009; 94: 4045-4052.
54. Tejera E, Plain A, Portelinha A, Caceres J, Rebelo I, Nieto-Villar J. Heart rate variability complexity in the aging process. J Comp Math Meth Med 2007;.18 (4): 287-296.
55. Safara F, Doraisamy S, Azman A, Jantan A, Ranga S. Wavelet Packet Entropy for Heart Murmurs Classification. Adv Bioinformatics 2012; 4: http://www.hindawi.com/jourNals/abi/2012/327269/cta
56. Darbin O, Dees D, Martino A, Adams E, Nariyoku D. An entropy-based model for basal ganglia dysfunctions in movement disorders. Biomed Res Int. 2013. 2013:5.10.1155/2013/742671
57. Wu, H-T, Lee C-Y, Liu A-B. Multiscale cross-approximate entropy analysis as a measurement of complexity between ecg r-r interval and ppg pulse amplitude series among the normal and diabetic subjects. Comput Math Methods Med 2013; 2013: http://dx.doi.org/10.1155/2013/231762
58. Dunn J, Hveem T, Pretorius M, Oukrif D, Nielsen B, Albergsten L et al.. Comparison of nuclear texture analysis and image cytometric DNA analysis for the assessment of dysplasia in Barrett's oesophagus. Br J Canc 2011;105(8): 1218–1223.
59. Kayser K, Borkenfeld S, Goldman T, Kayser G. How to measure diagnosis-associated information in virtual slides. Diagn Pathol2010.;6(11): http://www.diagnosticpathology.org/content/6/S1/S9.
60. Kayser K, Borkenfeld S, Goldman T, Kayser G. To be at the right place at the right time. Diagn Pathol 2011; 6 (68): http://www.diagnosticpathology.org/content/6/1/68.
61. Kayser K, Gabius H.J. The application of thermodynamic principles to histochemical and morphometric tissue research: principles and practical outline with focus on the glycosciences. Cell Tissue Res 1999; 3: 443-455.
62. Kayser K. Qantitive pathology in virtual microscopy: History, applications, perspectives. Acta Histochem 2013; 12: 179-188.

63. Luo L F, Molnar J, Dong H, Lu XG, Spengler G Attempts to introduce thermodynamics in anticancer therapy. Acta Sci Nat UnivIntramongol2006; 36: 295-303.
64. Luo L F, Molnar J, Dong H, Lu XG, Spengler G. Physicochemical attack against solid tumors based on the reversal of direction of entropy flow: an attempt to introduce thermodynamics in anticancer therapy. Diagn Pathol 2006; 1. (43): http://www.diagnosticpathology.org/content/1/1/43.
65. Molnar J, Thornton B, Molnar A, Gaal D, Luo L, Bergmann-Leitner E. Thermodinamic aspects of cancer: possible role of negative entropy in tumor growth, its relation to kinetic and genetic resistance. Lett Drug Des Discov2005; 2: 429-438.
66. . Vido J, Adam, R, Lorand-Metze K, Metze K. Computerized texture analysis of atypical immature myeloid precursors in patients with myelodysplastic syndromes: an entity between blasts and promyelocytes. Diagn Pathol 2011; 6; (93):http://www.diagnosticpathology.org/content/6/1/93.
67. Avtandilov G, Barsukov V. Informational analysis of immune and endocrine organs. Morphological changes in the course of infection. Zentralbl Pathol 1992: 138(5); 345-349.
68. Areshidze D, Timchenko L, Kozlova M, Syomin I, Rzhepakovsky I, Piskov I et al. Influence of Biologically Active Substance “STEMB” on a Morphofunctional Condition of a Liver and Kidneys of Rats at an Experimental Hepatorenal Syndrome. J Pharm Nutr Sci 2015; 5: 186-193.
69. Areshidze D, Timchenko L, Kozlova M. Information Condition of the liver of Dogs at Pathologies at the Reproductive Period of Ontogenesis. Acad J Canc Res 2013; 6(2): 79-83.
70. Areshidze D, Timchenko L, Kozlova M. Informational condition of Dogs liver at Pathologies. Global Vet 2013; 11 (3): 357-361.