Influence of Biologically Active Substance "STEMB" on a Morphofunctional Condition of a Liver and Kidneys of Rats at an Experimental Hepatorenal Syndrome

D.A. Areshidze[1,*], L.D. Timchenko[2], M.A. Kozlova[1], I.A. Syomin[1], I.V. Rzhepakovsky[2], S.I. Piskov[2] and V.N. Vakulin[2]

[1]*Researching Laboratory of Experimental Biology and Biotechnology, Moscow State Regional University, Moscow, Russian Federation*

[2]*Institute of Applied Biotechnology Department of the living systems of the North Caucasus Federal University, Stavropol, Russian Federation*

Abstract: In this investigation, we revealed that application of the biostimulating tissue preparation based on chicken embryos ("STEMB") at experimental hepatorenal syndrome showed improvement of the morphofunctional parameters of a liver. Under the influence of a biostimulator the relative mass of a liver considerably decreased and biochemical markers of a condition of a liver (total bilirubin, ALT, AST) returned to normal. Besides, under the influence of "STEMB" the tendency to normalization of mitotic, apoptotic and binuclear cells index of liver tissues and considerable decrease in a necrotic index is observed. Results of the research conducted by us allow to make a conclusion on possibility of inclusion of "STEMB" in a complex therapy of HRS for treatment of the injuries of a liver which are its main reason.

Keywords: Hepatorenal syndrome, liver, kidney, biogenic stimulants, tissue therapy, chicken embryo.

INTRODUCTION

Preparations on the basis of various tissues of an animal and plant origin are promising branch of pharmacology and find application in modern medical and veterinary practice. Preparations contain the natural composites peculiar for living cells or tissues and carry out stimulation in process of functional demand of an organism. They are a source of the primary nutrients, vitamins, macro and microelements [1-3].

Biologically active tissue preparations exist in the form of extracts, homogenates, cellular and subcellular suspensions, supernatants. Intact samples of tissues as well as samples processed in various ways may be used for preparation of bioactive substances.

The main active ingredients of the manifold group of tissue preparations having the wide list of raw sources are so called biogenous stimulators (or biogenic stimulants) which are group of the biostimulating substances with non-uniformon chemical structure. It is usually attribute to carry to them some low-molecular organic compounds, for example, the organic acids containing in living cells (di-carboxylic and tri-carboxylic acids, RNA, DNA, etc.) and low-molecular peptides [4].

One of the current directions of modern biotechnology is an obtaining of biogenous stimulators from cells, tissues and organs of eukaryotes, and also whole multi-cellular organisms [5].

In tissue therapy, various forms and methods of processing of an initial tissue substratum are applied to increase of its biological activity, bioavailability and improvement of pharmacological properties. For production of a complex of biologically active agents possessing a range of stimulating effects, living tissues often subject to preliminary stress-producing influence. The hypothermia, conservation, influence of weak ionizing radiation, laser irradiation, etc. can act as stressor, which promote a production of bioactive substances in tissue [6-7].

Application of the substances obtained as a result of stress-producing influence leads to increase of resistance of an organism to infectious agents and decrease in intensity of course of inflammatory processes, intensifies specific physiological functions of cells and tissues of an organism, stimulates growth and productivity.

The biogenous stimulators containing in tissue preparations have impact on the main parties of a metabolism that is expressed in change of metabolic and energy processes of an organism. Activating immune and regenerative functions of an organism, tissue therapy quite often is effective at smoldering pathological processes of various nature –

*Address correspondence to this author at 105005, Moscow State Regional University, Researching Laboratory of Experimental Biology and Biotechnology, Radio st. 10A, Moscow, Russian Federation;
Tel: +789096433756; Fax: +74986846918;
E-mail: Nihilist78@mail.ru, notbio@mgou.ru

ISSN: 2223-3806 / E-ISSN: 1927-5951/15

inflammatory, degenerative, atrophic, tumoral, etc. In modern medicine tissue therapy finds application at eye and skin diseases, arthronososes, in treatment of burn injuries and neoplastic processes [8-12]. In veterinary science and medicine tissue therapy is applied at some noncontagious and infectious diseases of farm animals, at nonhealing wounds, ulcers, some diseases of skin, lungs, etc.; as stimulators at sagination of young growth, for increase of dairy efficiency of cows and wool efficiency of sheep, for increase of fertility and decrease in mortality of young growth and breeding animals, especially for decrease in postnatal mortality [13].

Immunocompetent organs, a liver, a skin, muscles, placenta and chorion, separate tissues of an embryo of various species may serve an initial raw materials for obtaining of biostimulators. There are also substances for which preparation whole embryo completely is used [14-16].

High biological potential possess the supernatants of tissue extracts and homogenates, which contain biologically active agents with a low molecular mass (< 10 kD), but are exempted from high-molecular fragments of cellular organelles. Such substances possess high bioavailability and comprehensibility, representing the concentrated mix of biostimulators and nutrients [17].

One of promising sources for obtaining of tissue preparations is the chicken embryo [18-21].

One of the major target organs of action of biogenous stimulators is one of the most important organs of homeostasis providing – a liver.

Various diseases of a liver are among the most serious threats to health of the person in the modern world. Risk factors of development the pathologies are toxins of the environment and food, bacterial and virus infections, drugs, alcohol, etc. [22-27]. One of the most dangerous complications of pathological process in a liver is the hepatorenal syndrome (HRS), which is distinguished with high morbidity and mortality [28].

Hepatorenal syndrome is the potentially reversible disease, which is characterized by weakening of kidney function, the expressed violations of cardiovascular function and a hyperactivity of endogenous vasoactive systems. This sort of violations develops at patients with the expressed liver failure owing to an acute or chronic disease of a liver (hepatitis, hepatosis, cirrhosis), in the absence of any etiological factors of direct damage of kidneys [29-31]. In kidneys the syndrome manifested in the form of vasoconstriction becoming the reason of decrease in level of a glomerular filtration whereas in general blood circulation there is a decrease in vascular resistance because of an intraorgan and peripheral arterial vasodilatation [32-33].

It is usual to allocate two kinds of a hepatorenal syndrome: acute and chronic. It is supposed that division of the hepatorenal syndrome into types displays stages of one process. HRS of the I type develops acute, it is characterized by fast rates of increase of level of serum creatinine owing to prompt deterioration of kidney function. The I type HRS develops as a consequence of acute liver failure. This type of the syndrome is difficult reversible; the survival at HRS of the I type makes only 20%. Hepatorenal syndrome of the II type is characterized by the long, less clinically expressed development, which is a consequence of chronic diseases of a liver. For this type is peculiar a slowly progressing renal failure, development of refractory ascites and gradual increase of level of serum creatinine. The second type of hepatorenal syndrome is more widespread and noted with lower mortality rates and better responds to therapy [34-35].

As the reason of development of hepatorenal syndrome may serve viral and toxic hepatitis, an alcoholic liver disease and non-alcoholic fatty liver disease (non-alcoholic steatohepatitis), acute poisonings with hepatotoxic substances, liver cirrhosis, acute fatty hepatosis of pregnancy, bacterial peritonitis, neoplastic processes in a liver [36-37]. The risk factor promoting development of HRS is excess or excessively prolonged use of nonsteroidal anti-inflammatory drugs, which leads to inhibition of prostaglandin synthesis, decrease in a renal blood-flow and development of a renal failure [38].

Therapy of hepatorenal syndrome includes a complex of the actions directed on certain links of pathogenesis. Treatment of hepatorenal syndrome provides, first of all, elimination of its prime cause that is therapy of a liver failure. Besides, therapy of HRS means the actions promoting its reverse development by restoration of function of kidneys. Complexes of the preparations used in modern therapy of the hepatorenal syndrome are formed on set of the effects necessary for restoration of morphological and functional parameters of a liver, stabilization of hemodynamics and normalization of blood circulation in kidneys [39-41].

For specific therapy of the developing hepatorenal syndrome are applied various vasoconstrictors promoting improvement of kidney blood circulation and a glomerular filtration, increasing intravascular volume (vasopressin, ornipressin, terlipressin, norepinephrine, somatostatin, endothelin) [42-47].

In therapy of the liver failure, which is the prime cause of development of hepatorenal syndrome, are applied as specific therapy with hepatoprotective agents and the nonspecific adaptation therapy directed on restoration of morphofunctional integrity of an organism, increase of its nonspecific resistance and adaptation and regenerative potential of tissues and organs. Also ensuring is additional receipt of biogenous elements in an easily accessible form which is necessary for relief of regenerative and recovery processes in an organism in the conditions of the reduced metabolic function and oppression of immunological processes.

As the liver is the central link of a metabolism, including a metabolism of medicinal substances, it is important to apply the medicines having sufficient therapeutic effect at a low hepatotoxity and a minimum of side effects. In particular, at the therapy of diseases of a liver at all and peculiar the hepatorenal syndrome are successfully applied bioactive herbal preparations [48-53].

Hepatoprotective and total stimulating potential of various adaptogens of herbal and animal origin applied within so-called "tissue therapy" is very promising but a meanwhile low studied in therapy of hepatorenal disorders [54-55].

Thus, it represented to us actual to investigate an influence of a bioactive tissue preparation “STEMB”, made of embryonic tissues of chickens, on a morphofunctional condition of a liver and kidneys at an experimental hepatorenal syndrome.

The preparation “STEMB” is made of natural raw materials of an embryonic origin, contains an optimum set of biologically active agents: organic acids, vitamins, enzymes, hormones, macro and microelements, i.e. natural components peculiar for living cell or tissue which carry out stimulation in process of functional demand of an organism

In essence, “STEMB” is the preparation which exclusive properties are reached due to a complex of the manipulations directed on activation of biochemical processes in embryonic tissue that allows to increase the grade of concentration of biologically active agents in a substratum, including through formation of significant value of contain of biogenic stimulators. It should be noted that the "biogenic stimulators" containing in similar preparations have impact on the main parties of a metabolism. This influence is expressed in change of metabolic and power processes of tissues and organs ad an organism as a whole.

The tissue preparation “STEMB” is made by method of homogenization of specially processed incubated chicken embryos. For preparation of this substation the incubated hen's eggs at 9th-10th day of development are used. At this stage of development the optimum ratio of mass of an embryo and extra embryonic tissues is observed, and also immunocompetent organs and blood system of an embryo are already formed. For additional stimulation of development of an embryo, eggs are processed by the low-frequency laser in certain days of incubation that promotes increase in the size and mass of an embryo, and also the accelerated development of the listed organs and increase of enzymatic activity in egg. After the end of incubation (for the 10th day) eggs are located in the refrigerator at a temperature of 2-4 °C for 7 days that promotes development of biogenous stimulators in embryo tissues. Further the such prepared eggs are released from a shell, exposed to homogenization and preserved. The preparation received as a result of the described manipulations possesses a wide set of biologically active agents capable to carry out the biostimulating action on an organism of animals.

Complex chemical composition and structural features of this preparation allow to suggest probability of its positive influence on rather thin mechanisms of regulation in immune and also, closely related, the hormonal and metabolic status that may cause anti-inflammatory and homeostatic effects, capable to have the regulating impact on development of a hepatorenal syndrome and to reduce severity of its manifestations.

MATHERIALS AND METHODS

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.

Modeling of Hepatorenal Syndrome

For toxic hepatorenal syndrome obtaining animals were subcutaneously injected under a withers skin fold with a mixture of carbon tetrachloride (0.2 ml) and seed-oil (0.1 ml) in an amount of 20 injections performed every other day. Presumably, the hepatotoxic effect of $CC1_4$ is caused by damage to cellular structures of free radicals formed in the metabolism of this compound in the endoplasmic reticulum of the liver. Hepatotoxicity is a major manifestation of the action of carbon tetrachloride, whereas in conditions of oxidative stress generated free radicals can have a damaging effect on other organs of the digestive system, especially when ingestion CCl_4 in the human or animal body.

Treatment Design

Experiment on modeling and treatment of hepatorenal syndrome was carried out within 40 days. The 65 animals (Male Wistar Albino rats) were randomized and divided into three groups. Group I, including 20 animals, served as intact control. Animals in Group II (untreated control group), also in quantity of 20 animals, were injected with CCl_4 according to the above described scheme. Rats in Group III, in quantity of 25 animals, were also injected with CCl_4, but at the same time received hypodermic injections of "STEMB" in a dosage of 3 mg/kg.bw at 7th, 14th and 21st day of research (experimental group).

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.

Biochemical Analyses

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

Histopathological Analysis

Small portions of liver and kidney 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, necrotic and binuclear cells indexes in liver. At hematoxylin and eosin stained sections were determined mitotic and necrotic cells. At sections stained by methylene blue-azure II with afterstain by fuchsine were determined apoptotic cells. Visualization was performed using a microscope Nikon Eclipse 80i at 900 × magnification. Study was made for 5 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.

The mitotic index was calculated by the formula:

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

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

Necrotizing index 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.

Binuclear hepatocytes index (relative number of amitotic cells) was calculated by the formula:

$BI=N_b/N\times100\%$,

where N_b – number of binuclear cells, N - total number of cells in the test population. All studies was made for 10 fields of view on each section.

Morphometric Studies

All measurements were taken with use of image analyzer "Videotest" at hematoxylin and eosin stained sections. In digital microphotos of sections of experimental animal's kidneys by image analyzer "AxioVision" were defined area of a glomerulus, area of Bowman's capsule, area of glomerular space, width of a lumen of a proximal convoluted renal tubule and lumen of Henle's loop. Study was made for 10 fields of view on each section.

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.

RESULTS

As a result of the conducted researches it is established that as a consequence of development of a hepatorenal syndrome in animals of the Group II in comparison with intact animals there is a decrease in body weight, on thus the absolute and relative mass of a liver significantly increases. Body weight and weight of a liver of animals of the Group III differ from values of Group II unreliably, but the relative mass of a liver of animals in this group is considerable below, than in untreated control. At the same time, weight parameters of the animals treated with "STEMB" do not differ from parameters of intact animals (Table **1**).

In carrying out biochemical studies, we found that the modelling of hepatorenal syndrome results in a substantial increase of level of examined parameters. In Group II substantial increase of level of all studied biochemical parameters of blood serum concerning parameters of intact animals is observed. At Group III we also detected increase of level of uric acid and creatinine concerning values characteristic for intact animals, thus creatinine level practically coincided with untreated control values. However at the same time the level of the total bilirubin and AST in this group it is reliable below, than in untreated control group; the content of the total bilirubin, ALT and AST in blood serum of these animals doesn't differ from the values peculiar to intact rats (Table **2**).

At pathomorphological research of preparations of a liver and kidneys it is established that in intact control the histological picture of the studied organs complied with norm. In a liver of rats of the Group II we noted the phenomena of a vacuolization and granularity of cytoplasm, increase in number of the lymphoid elements testifying to development of inflammatory reaction, expansion of sinuses and Disse's spaces (a consequence of hypostases). As well as in a case with kidneys, similar changes with a lesser extent expressiveness are noted in a liver of animals of the Group III.

In the analysis of mitotic and apoptotic activity, and also at determining of number of necrotic and binuclear hepatocytes considerable intergroup distinctions are also found (Table **3**). In a liver of animal of Group II and Group III we note decrease of apoptotic and mitotic activity in comparison with intact animals, and also increase of quantity of cells in a condition of a necrosis at simultaneous reduction of number of binuclear hepatocytes. Thus, changes in a liver of the Group III exhibit parameters though the similar to control, but with considerably less expressed character.

In kidneys of animals of the Group II it were observed karyorhexis, necrosis of cells of tubules, interstitial edema, stratification of loops of glomeruli, compression and falling off of loops of glomeruli with the accumulating liquid, a cellular infiltration and some elements of regeneration of an epithelium of tubules. In kidneys of animals of the Group III we noted a similar picture (Table **4**).

In the analysis of the studied morphometric parameters of kidneys it is established that both in the

Table 1: Effect of "STEMB" on Weight Parameters of Rats

Groups	Body weight, g	Absolute mass of liver, g	Reliable mass of liver, %
I group (n=20)	209.30±5.60	7.60±0.37	3.62±0.30
II group (n=20)	181.40±9.10*	9.10±0.44*	5.02±0.37**
III group (n=25)	201.80±12.51	7.69±0.91	3.81±0.31

Hereinafter marked values significantly different from that of the intact group (* - p≤0.05, **- p≤0.005,***- p≤0.0005). The dark background markes the values reliably different from values of the Group II.

Table 2: Effect of "STEMB" on Biochemical Parameters of Blood Serum

Groups	Uric acid, mcM/l	Creatinine, mcM/l	Total bilirubin, mcM/l	ALT, U/L	AST, U/L
I group (n=20)	125.1±3.96	76.06±3.60	9.82±0.38	114.2±2.70	179.5±3.72
II group (n=20)	340.2±20.66***	115.8±8.60***	23.88±1.42***	168.8±22.1*	303.1±33.74***
III group (n=25)	154.7±9.38*	115.71±5.19***	10.74±1.40	148.51±25.98	190.11±37.18

Table 3: Effect of "STEMB" on Liver Tissue Parameters

Groups	Apoptotic index, %	Mitotic index, %	Necrotic index, %	Binuclear hepatocytes, %
I group (n=20)	2.79±0.2	6.31±0.3	0.81±0.1	6.31±0.30
II group (n=20)	0.27±0.043***	0.65±0.114***	54.47±4.10***	0.47±0.06***
III group (n=20)	1.18±0.11***	2.89±0.19***	2.28±0.20***	0.97±0.08***

Table 4: Effect of "STEMB" on Parameters of Kidneys

Groups	Area of capsule, mcm^2	Area of glomerulus, mcm^2	Area of capsular space, mcm^2	Lumen of proximal kidney tubule, mcm	Lumen of Henle's loop, mcm
I group (n=40)	3780±264	2475±180	1308±108	7.41±0.5	5.95±0.4
II group (n=40)	6003±480***	3920±235***	2085±130***	20.32±0.4***	18.75±1.9***
III group (n=50)	5033±115*	2975±221	2059±137***	13.38±1.21***	10.37±0.87***

Group II and in the experimental Group III there is an increase in all studied parameters, but thus differences from parameters of kidneys of intact animals have more expressed character in organs of the Group II.

DISCUSSION AND CONCLUSION

As a result of the research conducted by us it is established that use of biologically active substance "STEMB" at an experimental hepatorenal syndrome at rats leads to a certain effect concerning a morphofunctional condition of a liver and kidneys.

In the analysis of a morphofunctional condition of kidneys it is established that application of "STEMB" doesn't completely back out pathological changes in organs that is confirmed by the key HRS diagnostic parameter - the high level of creatinine. Thus, it should be noted that degree of an aberration of the morphological parameters characterizing kidneys is less expressed, than in control that is confirmed by results of histologic researches.

Concerning a liver the "STEMB" renders more expressed effect, than concerning kidneys. In particular, both absolute, and the relative mass of organ at the rats receiving preparation injections practically don't differ from parameters of intact animals. Despite certain pathological changes in organ, and also decrease of mitotic and apoptotic activity, and also decrease in number of binuclear hepatocytes against some increase in number of hepatocytes in a condition of a necrosis, the level of plasma total bilirubin, AST and ALT practically doesn't differ from the level of intact animals. These facts speak well for hepatoprotective action of "STEMB" at the experimental hepatorenal syndrome.

Thus, it is possible to say that application of "STEMB" at the experimental hepatorenal syndrome at rats has the expressed hepatoprotective effect. This fact is very important as severity of disease state at hepatorenal syndrome is defined by liver injury, and the violations from norm at a function of a liver are the major factor of mortality at HRS. For this reason, the liver is the main target at treatment of HRS as restoration of function of a liver leads to the reverse development of HRS.

The conducted research allows to claim that bioactive preparation "STEMB" can be used as an element of therapy both at prevention, and at treatment of HRS as a hepatoprotector and/or in combination with other 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] Davydov M, Krikorian AD. *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Araliaceae) as an adaptogen: a closer look. J Ethnopharmacol 2000; 345-393. http://dx.doi.org/10.1016/S0378-8741(00)00181-1

[2] Panossian A. Wilkman G. Effects of adaptogens on the central nervous system and the molecular mechanisms associated with their stress-protective activity. Pharmaceuticals 2010; 3: 188-224. http://dx.doi.org/10.3390/ph3010188

[3] 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. http://dx.doi.org/10.1016/j.ijbiomac.2015.02.050

[4] Pawar VS, Shivakumar H. A current status of adaptogens: natural remedy to stress. Asian Pacific J Trop Dis 2012; 480-490.

[5] Areshidze DA, Timchenko LD, Rzhepakovsky IV, Syomin IA, Kozlova MA, Sizonenko MN. Immunomodulatory influence of succinate extract of the Californian earthworms (Eisenia foetida). Cahiers des Sciences Naturelles 2014; 23: 2-12.

[6] 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. http://dx.doi.org/10.1111/j.1365-2621

[7] Kashiwagi S. Brauns T, Gelfand J, Poznansky MC. Laser vaccine adjuvants: History, progress, and potential. Human Vaccines and Immunotherapeutics 2014; 10: 1892-1907. http://dx.doi.org/10.4161/hv.28840

[8] Gomes A, Giri B, Alam A, Mukherjee S, Bhattacharjee P, Gomes A. Anticancer activity of a low immunogenic protein toxin (BMP1) from Indian toad (*Bufo melanostictus*, Schneider) skin extract. Toxicon 2011; 58: 85-92. http://dx.doi.org/10.1016/j.toxicon.2011.05.008

[9] Di Nicola V, Di Nicola R. Self-repair in degenerative joint disease. Curr Aging Sci 2012; 5: 273-278. http://dx.doi.org/10.2174/1874609811205030015

[10] Chakraborty PD, Bhattacharyya D., Zheng J. Aqueous extract of human placenta as a therapeutic agent. Recent Advances in research on the human placenta. Rijeka.: InTech 2012; 77-92. http://dx.doi.org/10.5772/31669

[11] Filardo G, Kon E, Di Martino A, Di Matteo B, Merli ML, Cenacchi A, Fornasari PM, Marcacci M. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. BMC Musculoskelet. Disord 2012; 13: 1-8. http://dx.doi.org/10.1186/1471-2474-13-229

[12] Shojaee M, Navaee F, Jalili-Firoozinezhad S, Faturechi R, Majidi M, Bonakdar S. Fabrication and characterization of ovalbumin films for wound dressing applications. Materials Science and Engineering 2015; 48: 158-164. http://dx.doi.org/10.1002/app.24537

[13] Florescu S. Paraschiv S, Rarinca C, Stavri J, Constantinescu E, Radu A, Georgescu D, Radu A. Effect of biogenic stimulants in the early fattening of young sheep. Lucrarile Stiintifice ale Institutului de Cercetari pentru Nutritie Animala 1975; 117-130.

[14] Tyagi JS, Merlino GT, de Crombrugghe B, Pastan I. Chicken embryo extracts contain a factor that preferentially blocks the accumulation of RNA polymerase II transcripts in a cell-free system. Journal of Biological Chemistry 1982; 257: 13001-13008.

[15] Yagi A. Bioactive constituents of a human placenta hydrolysate – from biogenic stimulants reported by Filatow to biological markers of collagen generation. Natural Medicines 2004; 58: 121-131.

[16] Huang D, Yang, Wang C, Ma S, Cui L, Huang S, Xu M, Sheng X, Weng Q. Immunostimulatory activity of protein hydrolysate from Oviductus Ranae on macrophage *in vitro*. Evidence-based Complementary and Alternative Medicine 2014; 2014: 1-11. http://dx.doi.org/10.1155/2014/180234

[17] Skehel JM. Preparation of extracts from animal tissues. Methods in Molecular Biology 2004; 244: 15-20.

[18] Oh TH, Markelonis GJ. Dependence of *in vitro* myogenesis on a trophic protein present in chicken embryo extract. Proceedings of the National Academy of Sciences 1980: 77; 6922-6925.

[19] Yadgary L, Wong EA, Uni Z. Temporal transcriptome analysis of the chicken embryo yolk sac. BMC Genomics 2014; 15: 690. http://dx.doi.org/10.1186/1471-2164-15-690

[20] Li X, Su Y, Sun J, Yang Y. Chicken embryo extracts enhance spleen lymphocyte and peritoneal macrophages function. Journal of Ethnopharmacology 2012; 144: 255-260.

[21] Alibardi L, Mlitz V, Eckhart L. Immunolocalization of scaffoldin, a trichohyalin-like protein, in the epidermis of the chicken embryo. The Anatomical Record 2015; 298: 279-287. http://dx.doi.org/10.1002/ar.23039

[22] Sturgill MG, Lambert GH. Xenobiotic-induced hepatotoxity: mechanisms of liver injury and methods of monitoring hepatic function. Clinical Chemistry 1997; 43: 1512-1526.

[23] Mudipalli A. Lead hepatotoxity & potential health effects. Indian. J Med Res 2007; 119: 1-2.

[24] O'Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease. Hepatology 2010; 51: 219-226. http://dx.doi.org/10.1002/hep.23258

[25] Schjott J, Mizuguchi Y (Ed.). Adverse effects of drugs and toxins on the liver. Liver biopsy in modern medicine. Rijeka.: In Tech 2011; 137-162.

[26] Kumar Seth R, Kumar A, Kumar Seth A, Chatterjee S. Environmental toxin-linked nonalcoholic steatohepatitis and hepatic metabolic reprogramming in obese mice. Toxicological Sciences 2013; 134: 291-303. http://dx.doi.org/10.1093/toxsci/kft104

[27] Ejtehadi A, Anushiravani A, Bananzadeh B, Geramizadeh F. Gastrointestinal Basidiobolomycosis accompanied by liver involvement: a case report. Iran. Red. Crescent. Med J 2014; 16: 1-5. http://dx.doi.org/10.5812/ircmj.14109

[28] Chang Y, Qi X, Li F, Wang S, Wang Z, Zhang Z, Xiao C, Ding T, Yang C. Hepatorenal syndrome: insights into the mechanisms of intra-abdominal hypertension. Int J Clin Exp Pathol 2013; 6: 2523-2528.

[29] Wong F, Blendis L. New challenge of hepatorenal syndrome: prevention and treatment. Hepatology 2001; 34: 1242-1251. http://dx.doi.org/10.1053/jhep.2001.2920

[30] Schepke M. Hepatorenal syndrome: current diagnostic and therapeutic concepts. Nephrol Dial Transplant 2007; 22: 2-4. http://dx.doi.org/10.1093/ndt/gfm656

[31] Assimakopoulos SF, Vagianos CE. Bile duct ligation in rats: A reliable model of hepatorenal syndrome? World J Gastroenterol 2009; 15: 121-123. http://dx.doi.org/10.3748/wjg.15.121

[32] Arroyo V, Guevara M, Gines P. Hepatorenal syndrome in cirrhosis: pathogenesis and treatment. Gastroenterology 2002; 122: 1658-1676. http://dx.doi.org/10.1053/gast.2002.33575

[33] Gordon CE. Hepatorenal syndrome. Hospital Medicine Clinics 2012; I: 62-68. http://dx.doi.org/10.1016/j.ehmc.2011.11.003

[34] Wadei HM, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: Pathophysiology and Management. Clin J Am Soc Nephrol 2006; 1: 1066-1079. http://dx.doi.org/10.1080/13651820701867935

[35] Kashani A, Landaverde C, Medici V, Rosarro L. Fluid retention in cirrosis: pathophysiology and management. Q J Med 2008; 10: 71-85. http://dx.doi.org/10.1093/qjmed/hcm12

[36] Mammayev SN, Karimova AM. Hepatorenal syndrome of the 1-st and 2-nd type: state-of-the-art. Russian Journal of Gastroenterology, Hepatology, Coloproctology 2008; 6: 4-11.

[37] Licata A, Maida M, Bonaccorso A, Macaluso FS, Capello M, Craxi A, Almasio PL. Clinical course and prognostic factors of hepatorenal syndrome: a retrospective single-center cohort study. World J Hepatol 2013; 12: 685-691. http://dx.doi.org/10.1002/hep.23286

[38] Davidson AM. Hepatorenal failure. Nephrol Dial Transplant 1996; №8: 24-31.

[39] Nadim MK, Kellum JA, Davenport A, Wong F, Davis C, Pannu N, Tolwani A, Bellomo R, Genyk Y. Hepatorenal syndrome: the 8th international consensus conference of the Acute Dialysis Quality Initiative (ADQI) Group. Critical Care 2012; 16: 1-17. http://dx.doi.org/10.1186/cc11188

[40] Blokhina NV, Pasechnik IN. The modern possibilities of the hepato-renal syndrome treatment. Surgery 2013; 8: 81-85.

[41] Low G, Alexander DJ. Hepatorenal syndrome: Aetiology, diagnosis, and treatment. Gastroenterology Research and Practice 2015; 2015: 1-11. http://dx.doi.org/10.1155/2015/207012

[42] Moore K. Endothelin and vascular function in liver disease. Gut 2004; 53: 159-161. http://dx.doi.org/10.1136/gut.2003.024703

[43] Wong F. Treatment of hepatorenal syndrome. Indian Journal of Gastroenterology 2006; 25: 8-12.

[44] Ng CKF, Chan MHM, Tai MHL, Lam CWK. Hepatorenal syndrome. Clin Biochem Rev 2007; 28: 11-17.

[45] Martin-Llahi M, Pepin M-N, Guevara M, Diaz F, Torre A, Monescillo A, Soriano G, Terra C, Fabrega E, Arroyo V, Rodes J, Gines P. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. Gastroenterology 2008; 134: 1352-1359.

[46] Nazar A, Pereira G-P, Guevara M, Martin-Llahi M, Pepin M-N, Marinelli M, Sola E, Baccaro ME, Terra C, Arroyo V, Gines P. Predictors of response to therapy with terlipressin and albumin in patients with cirrhosis and type 1 hepatorenal syndrome. Hepatology 2010; 51: 219-226. http://dx.doi.org/10.1177/2050640613502900

[47] Boyer TD, Sanyal TD, Boyer A, Garsia-Tsao A. Predictors of response to terlipressin plus albumin in hepatorenal syndrome (HRS) type 1: Relationship of serum creatinine to hemodynamics. J Hepatol 2011; 55: 315-321. http://dx.doi.org/10.1016/j.jhep.2010.11.020

[48] Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother Res 2007; 21: 17-25. http://dx.doi.org/10.1002/ptr.2023

[49] Areshidze DA, Timchenko LD, Klimenko AI, Gulykin MI, Kozlova MA. Influence of an enzymatic hydrolyzate of *Chlorophytum comosum (L.)* on morphofunctional integrity of a liver of white rats at experimental toxic damage during various periods of ontogenesis. Global Veterinaria 2013; 11: 794-802. http://dx.doi.org/10.5829/idosi.gv.2013.11.6.82147

[50] El-Gengaihi SE, Hassan EE, Hamed MA, Zahran HG, Mohammed MA. Chemical composition and biological evaluation of *Physalis peruviana* root as hepato-renal protective agent. Journal of Dietary Supplements 2013; 10: 39-53. http://dx.doi.org/10.3109/19390211.2012.760509

[51] Dkhil MA, Al-Quraishy MS, Diab MS, Abdel Moneim AE. The potential protective role of *Physalis peruviana* L. fruit in cadmium-induced hepatotoxicity and nephrotoxicity. Food and Chemical Toxicology 2014; 47: 98-106. http://dx.doi.org/10.1016/j.fct.2014.09.013

[52] Areshidze DA, Timchenko LD, Rzhepakovsky IV, Kozlova MA, Syomin IA, Piskov SI. Morphofunctional condition of hepatorenal system of rats at correction of the experimental CCl_4-induced hepatorenal syndrome using an enzymatic hydrolyzate of *Chlorophytum comosum L.* Bothalia 2015; 45: 70-80.

[53] Caglayan K, Gungor H, Cinar B, Avci B, Gur S, Arslan N. Effects of oleuropein on serum inflammatory cytokines and histopathological changes in rats with pancreatitis. Adv Clin Exp Med 2015; 24: 213-218. http://dx.doi.org/10.17219/acem/40453

[54] Fan Q-L, Huang C-G, Jin Y, Feng B, Miao H-N, Li W-J, Jiao B-H, Yuan Q-S. Effects of shark hepatic stimulator substance on the function and antioxidant capacity of liver mitochondria in an animal model of acute liver injury. Acta Biochim Biophys Sin 2005; 37: 507-514. http://dx.doi.org/10.1111/j.1745-7270.2005.00081

[55] Mobley CB, Toedebusch RG, Lockwood CM, Heese AJ, Zhu C, Krieger AE, Cruthirds CL, Hofheins JC, Company JM, Wiedmeyer CE, Kim DY, Booth FW, Roberts MD. 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. Nutrition & Metabolism 2014; 11: P. 1- http://dx.doi.org/10.1186/1743-7075-11-19

Received on 11-06-2015 Accepted on 10-08-2015 Published on 25-08-2015

DOI: http://dx.doi.org/10.6000/1927-5951.2015.05.03.3