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Research Article

Protective Effect of Enzymatic Hydrolyzate of *Chlorophytum comosum (L.)* against Experimental Toxic Liver Injury in Wistar Rats at the Age of Nine Months

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Abstract

Investigation of the influence of the enzymatic hydrolyzate of *Chlorophytum comosum (L.)* on the liver of rats at the age of nine months at its experimental toxic damage showed that the substrate has expressed a pronounced hepatoprotective effect. Under his influence in the rat liver morphological changes induced by CCl_4 is much less pronounced than in the controls. It is also less significant deviations from normal levels of Alanine transamilase (ALT), Alanine transaminase AST and Total Bilirubin. Information analysis of the state of the body indicates that the level of adaptation and regeneration opportunities of the liver of rats treated with an enzymatic hydrolyzate of *Chlorophytum comosum* (L.), significantly higher than that of the liver of rats with experimental toxic liver damage without the use of test substrate. Histopathological analysis confirmed the alleviation of liver damage and reduced lesions caused by enzymatic hydrolyzate of *Chlorophytum comosum (L.)*

1. INTRODUCTION

Liver disease is one of the most pressing public health problems around the world, because the liver is one of the central bodies to ensure homeostasis. Hepatic injury is associated with distortion of various metabolic functions[1-4].

In modern scientific literature there are few reports about the healing properties the plant *Chlorophytum comosum* (L.). It is shown that the leaves of the plants have a high sorption characteristics with respect to formaldehyde, carbon monoxide, benzene, trichlorethylene, phenols and other compounds [3,4]. By chemical analysis of the enzymatic hydrolyzate of Chlorofitum comosum in its composition was found DL - ornithine monohydrochloride having disintoxicational and hepatoprotective action[7-14].

These facts allow us to consider this hydrolyzate as a biologically active substance having hepatoprotective properties, which allows authors to keep focused on the study of the effect of the hydrolyzate regenerative potential of the mammalian liver. To test the hypothesis about the effectiveness of bio-stimulation, we carried out a study whose purpose was to examine the severity of liver damage while taking CCl_4 enzymatic hydrolyzate of *Chlorophytum comosum* (L.).

These facts allow us to consider this hydrolyzate as a biologically active substance having hepatoprotective properties, which allows authors to keep focused on the study of the effect of the hydrolyzate regenerative potential of the Male Wistar Albino rats liver in old age. To test the hypothesis about the effectiveness of bio-stimulation, we carried out a study whose purpose was to examine the severity of liver damage while taking CCl_4 enzymatic hydrolyzate of *Chlorophytum comosum* (L.).

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2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh aerial parts of the *Chlorophytum Comosum* plant were collected in Moscow state regional University botanical garden. The collected plant samples were washed thoroughly with running tap water and were used to prepare an enzymatic hydrolyzate.

2.2 Preparation of Hydrolyzate

The starting substance in an amount of 0,333 kg was washed thoroughly under running tap water (previously placing in gauze). The washed raw material was placed in a glass container with 1.0 liter (1:3) of tap water heated to a temperature of (45 ±1) ◦C. Na_2CO_3 was added to the mixture to pH 8.2-8.3 (pH was defined on phenolphthalein). Then were added 0.15 kg of crushed pancreas of cattle. Then was added of content. Then container was covered by tightly cotton-gauze pad with parchment and placed in a heat chamber at (45 ±1) ◦C. Kept for 10 days, shaking during the first day every 15 minutes to 5 minutes, and in the following days every two hours to 5 minutes. The dynamics of the enzymatic process control from the increase of the content of amino nitrogen. For the ninth or tenth day increase stops and hydrolyzate leave for the night in the switched-off heat chamber. Then the hydrolyzate was filtered through a filter paper. In the filtrate was added chloroform in the ratio 2% to the total amount, the substance was placed in a glass flask with the rubber stopper and was stored at a temperature from 2 to 8 ◦C.

2.3 Animals

Male Wistar Albino rats of body weights ranging from 150 to 200 g were used in the study. Age of the animals was nine months. 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.

2.4 Toxity Studies

Based on previous studies[15, 16], the dose of enzymatic hydrolyzate of *Chlorophytum comosum* (L.) at the concentration of 6 mg/kg.bw was chosen for the experiments.

2.5 Treatment Design

270 animals (Male Wistar Albino rats) were randomized and divided in to three groups of ninety animals each. Group I served as intact control. Animals in Group II were inhaled by carbon tetrachloride to 2 min. per day for 6 days (control group). Rats in Group III were inhaled carbon tetrachloride to 2 min a day for 6 days, but at the same time treated with drinking enzymatic hydrolyzate of *Chlorophytum comosum* (L.) at the concentration of 6 mg/kg.bw (experimental group).

Selection of carbon tetrachloride (CCl_4) as an agent acting on the liver, due to the fact that the substance is a direct liver poison, widely used in experimental medicine and biology. Selecting the liver-toxic and exposure is determined by the fact that the use of carbon tetrachloride under this scheme provides the appearance and development of reversible changes in liver tissue and organ level.

2.6 Assessment of Hepatoprotective Activity

2.6.1 Biochemical Estimation

For the 7th day of experiment, blood samples were collected by direct cardiac puncture using light ether anesthesia. Blood was separated by centrifuging at 2500 rpm for 20 min and used for analysis of AST, ALT, and Total Bilirubin by using standard Kits (PLIVA-Lachemia Diagnostica, Chech Republic, Brno).

2.6.2 Histopathological Analysis

A small portion of liver was taken and fixed in to 10% formaldehyde. After several treatments for dehydration in alcohol, sections having 5µm thickness were cut and stained with hematoxylin and eosin and histopathological analysis was carried.

To detect apoptotic cells semi-thin sections (3µm) were stained with methylene blue-azure II with afterstain by fuchsin. All stained sections were embedded in balsam.

2.6.3 Determination of Mitotic, Apoptotic and Necrotic Index

At hematoxylin and eosin stained sections were determined mitotic and necrotic cells. At sections stained by methylene blue-azure II with afterstain by fuchsin were determined apoptotic cells. Visualization was performed using a microscope Nicon 500L at 900 x magnification. Studied was made for 5 fields of view on each section.

Apoptotic index was calculated by the formula:

$$AI=N_a/N,$$

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

The mitotic index was determined by the formula:

$$MI=N_m/N,$$

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

Necrotizing index calculated by the formula:

$$NI=N_n/N,$$

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

2.6.4 Morphometric Studies

Volume of the nuclei of hepatocytes was measured by image analyzer "Videotest" at hematoxylin and eosin stained sections.

2.6.5 Studies of the Information Condition of the System of the Liver

Carried out a breakdown of the aggregate of the measured volumes of hepatocyte nuclei into classes.

Based on the concept of information in a tissue system, like 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). In this case, the baseline characteristics, which were used to calculate these parameters, can vary widely (the linear dimensions of the structures, their number, etc.). In our study was defined the volume of the nuclei of hepatocytes.

Information morphological capacity H_{max}, which means the maximum structural diversity, 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.The calculation was made using the formula:

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

Where ΣP_i is the sum of probabilities of stay 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 maximum 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 is 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:

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

High levels of relative morphological entropy provide evidence of the disorder of the system and significantly reducing of its structural integrity [5].

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

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

With these data, the researchers have the opportunity to calculate the equivocation of the system (the value of reliability) D:

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

Where H_n - real structural diversity in normal, H_p - real structural diversity in pathology[17].

2.7 Statistical Analysis

Values are expressed as mean (± SD). The statistical analysis was performed using one-way analysis of variance (ANOVA). The statistical difference determined using repeated measures analysis of variance or paired Student *t*-tests. A p value of < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effects of Enzymatic Hydrolyzate of *Chlorophytum comosum* (L.) on Histopathology

At the pathomorphologic examination of the liver of rats exposed to carbon tetrachloride was found by us that the body of animals had a red color, sometimes with yellow or gray tint. About 28% of rat liver was spotty. The body loose, easily torn, the cut oozing blood. The histological study noted a pronounced hepatic diskomplexation beams. Hepatocytes were swollen and their cytoplasm is cloudy, the boundaries of the cells is not clear, the kernel is also swollen, bright, with blurred outlines. In hepatocytes clearly observed clear vacuoles. When stained with Sudan-III in 58% of the months in the vacuoles of hepatocytes revealed lipids. In rat liver hepatocytes detached state granular dystrophy. The vessels of the liver in different parts of the cut unevenly expanded and filled with blood, in the field of triads and signs of mild perivascular mesenchymal reaction.

In a number of cases are reported from connective tissue layer, significant in the field of triads are infiltrated with small cells thickened. Blood vessels (central vein capillaries) in the liver extended (hyperemia of blood vessels), the permeability of the walls of the blood cells is increased, marked focal hemorrhage. Among the cells of a large number of white blood cells, the macrophages. In hepatocytes, a large number of vacuoles, including lipid, as evidenced by coloring with Sudan - III. The individual cells are very large and in fact a continuum vacuole

In 77% of cases showed multiple foci of necrosis in different sizes, in which the structural elements of the individual cells are not rendered, and the liver tissue is a homogeneous structureless mass. In 45% of cases are reported extensive necrosis.

The observed changes indicate the development of animal subgroups typical toxic liver disease. However, some rats were established characteristic of the micro-focal alterative inflammation. A significant proportion of rats marked picture of acute toxic hepatitis with high intensity of tissue damage (hepatitis alterative). Some animals with severe steatosis is defined necrotic component.

The use of the hydrolyzate of comosum the simultaneous inhalation of CCl_4 in the liver that pathological changes in the body are much less severity. Thus, in the liver of all animals, and beams of the traces lobed structure. In this case, a few pockets of malnutrition alternate with areas represented with dual-core and intact hepatocytes (signs of recovery) or hepatocytes are able to start-up phase granular dystrophy, fatty degeneration occurs in 20%. Also, substantially less able hepatocyte necrosis. Noted the absence of focal hemorrhages, capillaries moderately bloodshot, and there are no signs of swelling, and reduces the permeability of which are registered in the group without the use of the hydrolyzate. Vessels in the triads moderately dilated. In this case, 17% of hepatocytes marked small vacuoles.

3.2 Effects of Enzymatic Hydrolyzate of *Chlorophytum comosum* (L.) on MI, AI and NI

For the liver of intact rats we found the MI equal to 6.95±0.52%, AI – 2.85±0.26%, and NI was 0.81±0.08%. The value of MI in the liver of animals of the control group was 1.20±0.14%, AI – 1.50±0.14% NI–8.2±0.33%. Application enzymatic hydrolyzate of Chlorophytum comosum (L.) in experimental toxic injury of the liver leads to the MI of 5.40±0.35%, AI – 2.20±0.22%, NI – 6.15±0.41%. (Fig. 1).

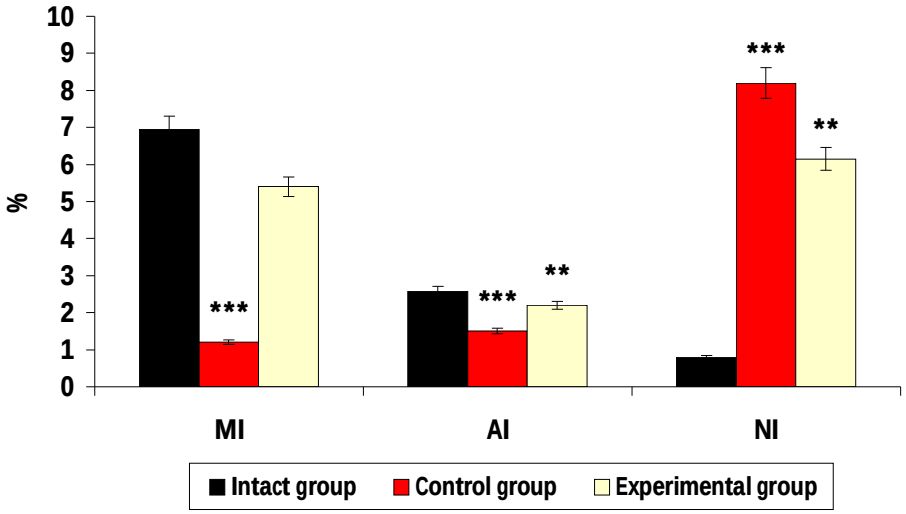

Fig. 1. The value of MI, AI and NI in the liver of rats. Values are significantly different from intact group. (*** indicates P<0.001, ** indicates P<0.01, * indicates p<0.05).

3.3 Effects of Enzymatic Hydrolyzate of *Chlorophytum comosum* (L.) on AST, ALT and Total Bilirubin Levels

ALT levels in the serum of rats of the intact group was 2.21±0.08 IU/L, AST level was equal 1.50±0.013 IU/L. Application hydrolyzate of *Chlorophytum comosum* (L.) in toxic liver damage leads to a substantial reduction of ALT (2.63 ± 0.1 IU/L in the control versus 2.20±0.1 IU/L in the serum of rats treated with the hydrolyzate) and AST (2.38±0.06 IU/L in control versus 1.61±0.10 IU/L in the serum of rats treated with the hydrolyzate). Total Bilirubin in serum of control rats was 6.82±0.19 IU/L, decreased bilirubin content in the serum of the experimental group animals to 7.10±0.10 IU/L at 14.02±0.1 IU/L in the control (Fig. 2).

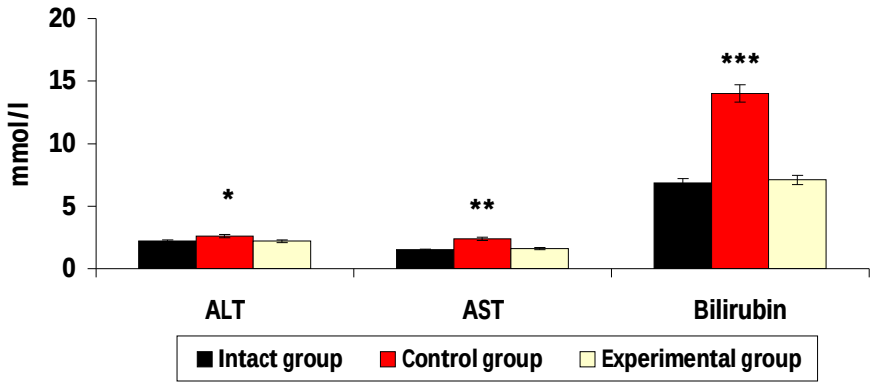

Figure 2: The value of H, S and h in the liver of rats. Values are significantly different from intact group. (*** indicates P<0.001, ** indicates P<0.01, * indicates p<0.05).

3.4 Effects of Enzymatic Hydrolyzate of *Chlorophytum comosum* (L.) on Informational Condition of Liver

The liver of rats of the intact group at the age of nine months was characterized by H equal to 2.483±0.018 bits, S amounted to 0.8822±0.018 bits, h is equal to 0.7343±0.005 bit, R amounted to 26.57±0.55% (Fig. 3, 4).

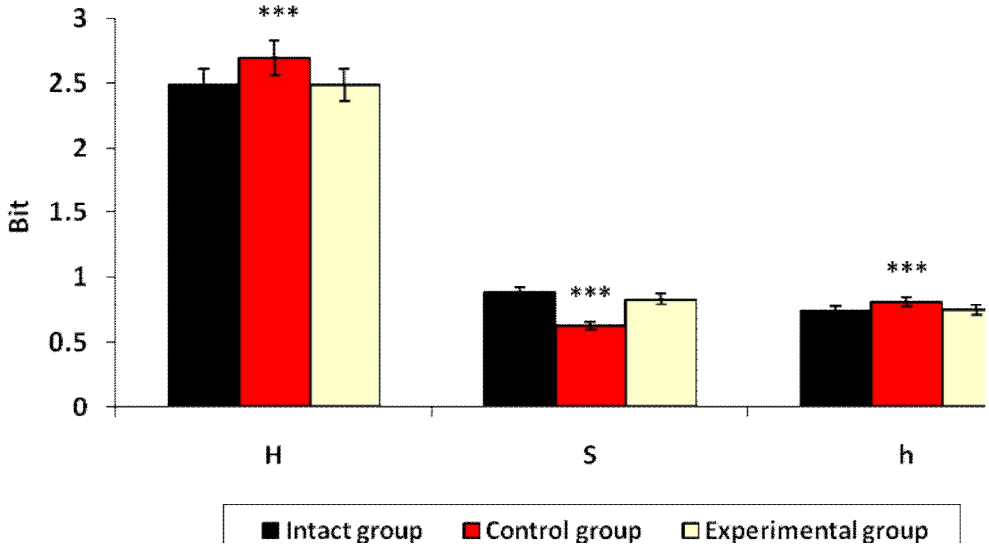

Figure 3. The value of H, S and h in the liver of rats. Values are significantly different from intact group. (*** indicates P<0.001, ** indicates P<0.01, * indicates p<0.05).

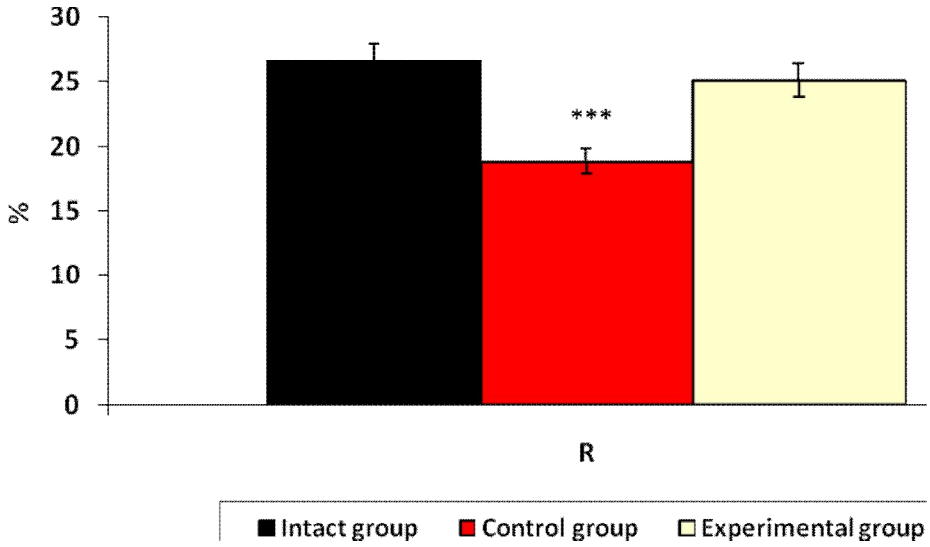

Figure 4. The value of R in the liver of rats. Values are significantly different from intact group. (*** indicates P<0.001, ** indicates P<0.01, * indicates p<0.05).

Information indicators of liver of rats being exposed to CCl_4, differ significantly from the age norm. Thus, H is 2.694±0.033 bits, S is equal to 0.6257±0.033 bits, h–0.8115±0.007 bits, R – 18.85±0.98%, ***e*** amounted to 0,211±0,011 bit.

The liver of rats at the age of nine months treated with the enzymatic hydrolyzate in parallel with inhalation CCl_4 is characterized by information parameters differ from the livers of rats inhaled carbon tetrachloride. H was 2.487±0.022 bits, S was 0.8333±0.022 bits, h– 0.749±0.007 bits, R is equal to 25.10±0.68%, e was 0.04±0.0002 bit.

4. CONCLUSION

The outcome of present investigation undoubtedly indicate that the treatment with fermentative hydrolizate of *Clorophytum comosum* was effective on inhibiting the hepatotoxicity induced by CCL_4 in vivo models, most likely because of content of DL - ornithine monohydrochloride or specific constituents present in the hydrolyzate.

Studies suggest that enzymatic hydrolyzate of *Clorophytum comosum* hooded has significant hepatoprotective properties, reduces the intensity of the inflammatory process. Pronounced positive effect of the hydrolyzate on liver regeneration, as evidenced by differences in the mitotic, necrotic, apoptotic index and the proliferation rate in the experimental groups. The liver of rats treated with an enzymatic hydrolyzate of Chlorofitum comosum at toxic damage, based on analysis of the information state body, is characterized by a high level of adaptation and regenerative capacity than the liver of rats of the first experimental group.

The study showed that in contrast to the results obtained under analogous conditions on young rats studied parameters liver rats

after applying the enzymatic hydrolyzate although they differ significantly from the indices of the control group of rats, as well and significantly different from the indices in intact animals.
We planned to identify more precisely the lead components responsible for hepatoprotective activity and to unveil the molecular mechanism behind its therapeutic action.

5. ACKNOWLEDGEMENTS
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