

*Materials of Conferences*

**INFLUENCE OF 20 % SPIRIT EXTRACT  
PRODUCED FROM DEAD BEES  
ON THE LEVEL OF HEPATOCYTES'  
PROLIFERATIVE AND APOPTOTICAL  
ACTIVITY DURING A STRESS**

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Liver plays an important role in homeostasis maintenance. The organ takes part in its maintenance both in normal processes supporting a life, and in adaptation of an organism to any loadings and stresses. The morphological and functional condition of this organ can indirectly reflect a condition of an organism as a whole [5]. Any change of such parameters as the linear sizes of a cell nucleus and a number of hepatocytes is one of typical signs of adaptation process taking place as the sizes of a liver cell nucleus directly depend on the amount of DNA in it [7]. The excitation or damage condition of hepatocytes can be accompanied by more intensive protein synthesis. The same phenomenon is also observed at the increase of functional activity of an organism as a whole. Moreover, it is possible to estimate a functional condition of body according to the linear sizes of hepatocytes' nucleus. Besides, it's typical of a liver to have rather constant correlation of diploid, tetraploid and polyploid hepatocytes. Any change of this correlation shows some functional disorder in the organ which can be caused by various damaging factors. The total number of cells and a correlation of various cellular populations in a normal condition is supported by the interaction of mitosis, amitotic division and apoptosis mechanisms [1].

One of ways to increase the adaptation abilities of separate organs, their systems and those of an organism as a whole is to apply biologically active additives (BAA). BAA's supply (manufacture) is an important branch of modern biotechnology [3,4].

BAAs produced from dead bees take an important place among other additives made from other products of beekeeping. Dead bees continue to possess high biological activity that results from the components building their structure such as: chitin, melanin, heparoids and other biologically active substances (BAS). These preparations (BAAs) possess adaptogenic (adaptation-stimulating), immunomodulatory, hepatoprotective, recycling and antitoxic properties [6].

Proceeding from the above-stated, the research of the influence of an extract from dead bees on a liver during a chronic or sharp stress seemed to us quite promising.

Vistar rats, both male and female, at the age of 1,5 months were chosen to make a set of experiments. The first group of animals (n=20) was a control one, the second group (n=20) was an experimental one, in the latter group all rats received an extract of dead

bees with a drink, its amount being calculated according to a rat's weight, i.e. 0,1 g of an extract = 100 g of weight. Both groups were subdivided into 2 subgroups. All the animals were kept in the standard laboratory conditions.

Within three weeks a chronic stress was provided in one of the subgroups of each group. Two other subgroups were subject to an immobilization stress. A chronic stress consisted in using a test of forced swimming which was carried out by means of the standard technique [2]. The rats of a control and experimental groups were forced to swim until complete exhaustion once a week throughout 3 weeks, after that the sampling of liver cells for morphological and histochemical analysis was taken. The experiments on animals were carried on according to the regulations of protection of the vertebrate animals used in the scientific purposes (Regulations and recommendations for the European independent committees concerning ethics, Brussels, 1995, 1997; Recommendations to Committees on the ethics, making examination of biomedical research, Geneva, 2000).

An immobilization stress was simulated by fixing rats, with their paunches up, on a laboratory restraining chair for an hour, then the animals were put down.

Histologic research was carried out by the standard technique with samples dyed by hematoxylin and eosin, with the subsequent calculation of mitotic and apoptotic indices (MI and AI) being made. These indices were calculated according to a number of hepatocytes under mitosis or apoptosis conditions in 1000 cells.

As a result, it has been established that the signs of the initial stage of a granular dystrophy were observed in the liver of all rats in the control group. The micronecrosis process was registered. The hepatocyte borders were blurred, they could not be accurately distinguished. The joist (beam) structure was damaged in some places. The morphological analyses result of rats' liver in the experimental groups corresponded to the standard results under normal conditions.

MI for the rats in the control group subject to a chronic stress appeared to be the least one ( $1,175 \pm 0,08 \%$ ) and evidently differed from the result (index) in the experimental group ( $2,00 \pm 0,1 \%$ ) and from MI of the immobilized rats' liver in the control group ( $2,23 \pm 0,12 \%$ ).

The similar tendency has been traced as far as AI is concerned: in the control group at a chronic stress AI was equal to  $0,025 \pm 0,001 \%$ ; in the experimental group under a chronic stress it amounted to  $0,04 \pm 0,005 \%$  that evidently differed from the previous result. In the control group subject to a sharp stress AI was equal to  $0,2 \pm 0,06 \%$  that appeared to doubtfully be higher than in the experimental group –  $0,17 \pm 0,03 \%$ .

Karyogram of rats' hepatocytes in both control groups has clearly shown 3 peaks with the displace-

ment tendency to the right that is characteristic for stress reactions which is accompanied by the increase of hepatocytes. In a liver of the experimental rats the karyogram had a more smoothed character, with 2 peaks being distinguished without any doubt. In a whole, the karyogram was displaced to the left. It is necessary to mention also that the karyogram for both groups which were under the conditions of a chronic stress was displaced to the left as compared to the immobilized rats' karyogram.

The spirit extract produced from dead bees stimulates the process of regeneration of hepatocytes population by means of a mitosis under both chronic and sharp stresses. In addition, AI in liver of the rats that were given the dead bees extract proves to be higher alongside a better pathomorphological result. This can be explained by the fact that under some stressful factors hepatocytes originally perish due to apoptosis, and only after that one can see both monocellular and local necrosis in the organ[8]. Taking into account the above, we can come to the following conclusion that the spirit extract produced from dead bees possesses a certain activity stimulating stress adaptation processes in the liver.

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The work was submitted to international scientific conference «Prospects for the development of university science», Dagomys (Sochi), September 21-24, 2009, came to the editorial office on 20.07.2009.

### THE PRACTICALLY HEALTHY PEOPLE'S LARGE INTESTINE MICROBIAL FLORA COMPOSITION

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At present, the normal microflora is, practically, being considered, as the owner's human organism integral part, the original and the peculiar extracorporeal human organ, having involved just into the proper and also the allogenic and the foreign substances, just into the structure, which the first is, practically, being involved just into the absorption, and, through which the translocation is being proceeded on, as the useful, well as the potentially harmful agents, including the microbial origin [3].

The practically healthy people's at the age in the range from 3 up to 45 years microbial flora has been studied and thoroughly investigated by us. The microscopic and the culture methods use have been shown, that the protozoa infection has been made up 2,73 % (e.g. 3 persons) just in this group, and this, moreover, is, considerably, being lowered the people's with the gastrointestinal tract pathologies analogous index. So, in all these cases, the protozoa finding has been accompanied with the bowels microflora disbiotic changes, having, at the same time, the various degree of the manifestation.

The «normal» microflora notion has already been defined, as the large intestine bacterial dissemination average physiological value, having taken into consideration the received final results' oscillations.

All these investigations have been carried out, having defined the qualitative and the quantitative compositions of the bacterial communities with the following space structure identification and also the ecological groups' hierarchy.

The received final data analysis has been permitted to be identified the 390 microorganisms strain and also to be related them to the 11 deliveries.

The bowels microflora composition study and the thoroughly investigation had already been shown, that its composition was enough diverse and suffice various (table 1). The obligate – anaerobic flora analysis was been shown, that the bowels' colonization density by the obligate – anaerobic bacteria had been presented by the representatives' high level concentrations, having been studied and thoroughly investigated taxa. So, the bacteroides' values oscillations have been from  $10^8$  up to  $10^{10}$  QEU/gr. The clostridia's dissemination lower bound has not been exceeded  $10^5$  QEU/gr. Thus, the healthy man's average colonization density by the obligate – anaerobic microorganisms has been defined, as 1gr.  $10,3 \square 0,7$  QEU/gr.