Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 4, 2013, pp. 495-498


# THE COMPARATIVE BIOCHEMICAL ASSESSMENT OF EVIDENCE OF ANTI-HYPOXEMIC ACTION OF ROYAL JELLY AND COMPOSITIONS WITH IT

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**ABSTRACT**. The beekeeping products containing various biologically active components are interesting enough to include them in an experimental research at an assessment of possible anti-hypoxemic and anti-oxidant effects.

**Keywords:** hypoxia, biologically active products, lipid peroxidation, anti-hypoxemic effect, anti-oxidant effect

## INTRODUCTION

In experimental pharmacology the model of a "high-rise" hypoxia is widely applied to an assessment of existence and evidence of antihypoxemic activity at means which are close in their properties - those of the analyzed apikompozitions (Kolchinskya A.Z., 1993). Unlike a normal baric and hyperbaric hypoxia it allows to characterize the anti-ischemic action to a great extent. In the given article one of variants of this model is used – that of an acute hyperbaric, or hypoxemic hypoxia which was caused by a single 6-hour exposition of laboratory animals in an altitude chamber of 0,125 cubic metres bulk with a supply-and-exhaust ventilation under pressure equal to ascending on the height of 7500-8000 metres. These conditions predetermine the development of changes in an organism, which, according to A.Z. Kolchinskaya's classification (1983), correspond to the III stage of a hypoxemic hypoxia and which are designated as an expressed hypoxia with an advancing decompensation. The modulation of its characteristics by the analyzed chemical agents serves as a basis for an assessment of their anti-hypoxemic effects.

## MATERIALS AND METHODS

The experiments are made on the sexually mature non-linear male rats weighing 180-240 g., kept in the general conditions of a vivarium. The analyzed chemical agents were injected into a laboratory animal in daily doses: native royal jelly (RJ) - 10 mg/kg, APITONUS (APTS: 2% of royal jelly, 98% of honey) - 500 mg/kg, APITOK (APT: 2% of royal jelly, 1% of propolis, 97% of honey) - 500 mg/kg, APIFITOTONUS-1 (APFT-1: 2% of royal jelly, 4% of pollen, 94% of honey) - 500 mg/kg, APIFITOTONUS-2 (APFT-2: 2% of royal jelly, 20% of pollen, 78% of honey) - 500 mg/kg during 10 days before placing the animals to the altitude chamber. Right after the extraction of the animals from the camera they were anaesthetized with ether and biosubstrata was sampled – i.e., blood, tissues of myocardium, liver and brain in which the researchers defined a complex of characteristics including: the indices of gas structure (partial pressure of oxygen and carbon dioxide - pO2 and pCO2) and acid-base balance (pH, concentration of HCO3 - an ion) of blood; common laboratory biochemical indices of blood serum, the changes of which steadily accompany the development of an acute hypoxemic hypoxia (Lukyanchuk V.D., et al., 1993): the activity of aminotransferases (nuclear heating plant and ALT), alkaline phosphatase, concentration of the general protein, general cholesterol, glucose, urea, potassium, sodium; the indices characterizing the condition of lipid peroxidation, one of the inducers of which is hypoxia (Vladimirov Y.A., 1987, Kolchinskya A.Z., 1993, Pozharov V.P. et al., 1993, Adaptation Medicine, 2008.): the concentration of malonic dialdehyde (MDD), the activity of NADF-N-POL and ASK-POL in tissues of myocardium, liver and brain.

## RESULTS AND DICUSSIONS

A six-hour stay of the rats in the hypoxemic camera with the residual pressure corresponding to the ascending on the height of 8000 metres was accompanied by the moderate but statistically reliable change of all four indices of gas structure and acid-base balance. The majority of characteristics of acid-base balance and gas composition of blood (with the exception of pCO2) haven't changed significantly after the prescription of royal jelly and apicompositions containing it and influence of hypoxemic factors in comparison with the corresponding indices of the intact rats. It probably can be regarded as "external" manifestation of protective effect of the prescribed apicompositions to a hypoxia. For a more precise comparative assessment of evidence of this action it was important to study the dynamics of a complex of biochemical indices of blood and the characteristics of the level of lipid peroxidation in various tissues.

The quantitative values of the activity of serum enzymes of the intact animals' blood (nuclear heating plant - 1,34 + 0,07 mmol / л*ч, ALT - 1,11 + 0,05 mmol / л*ч, alkaline phosphatase - 1,38 + 0,08 mmol / л*ч), and also the concentration of substrata (glucose - 9,73 + 0,32 mmol/l, the general cholesterol - 1,74 +

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Article received: August 2013; published: November 2013

0,08 mmol/l, the general protein - 83,3 + 1,7 g/l, urea - 6,05 + 0,26 mmol/l) and electrolytes (potassium - 5,28 + 0,16 mmol/l, sodium - 140,1 + 0,07 mmol/l) have significantly changed after the modelling of an acute hypoxemic hypoxia. In particular, the activity of ALT and AST has arisen: Nuclear heating plant - to 2,23 + 0,07 mmol / л*ч (166% from control), ALT - to 2,34 + 0,12 mmol / л*ч (211%) that, most likely, reflects the increased transmissivity of membranes in hepatocytes in the conditions of hypoxia. Sufficiently expressed hypoglycemia (7,11 + 0,17 mmol/l, or 73% from control), is consequent of both the intensive transition of glucose from blood to tissues where the need for it as an energy substratum considerably increases in the conditions of hypoxia, and the decrease of synthetic function of liver. The revealed hypoproteinemia (54,3 + 1,5 g/l, or 65% from control) and hypocholesterolemia (1,00 + 0,08 mmol/l, or 58%) can also be connected with the latter. The fixed increase of activity of alkaline phosphatase (2,32 + 0,09 mmol/l, or 168%) could be connected with the depression of one more liver function – that of biliary excretion. Diselectrolyte changes (hypokalemia - 4,03 + 0,05 mmol/l, or 71% and hyponatremia - 126,6 + 0,8 mmol/l, or 90%), most likely, reflect the renal "answer", in particular, in the form of violation of the reabsorbing processes, to the influence of hypoxemic factors. Finally, urea accumulation up to 160% from a reference level (9,68 + 0,55 mmol/l) at rats of the hypoxia series can be connected with the catabolism intensification of nucleic acids and the inclusion of the releasing purine bases into the comprising urea.

The presented changes of a complex of the common laboratory biochemical indices caused by the placing the laboratory animals into the hypoxemic camera, were different in cases of preventive prescription of RJ and apicomposition. Thus, the activity of nuclear heating plant of the rats receiving native royal jelly authentically decreased in comparison with the hypoxia series to 1,91 + 0,05 mmol / л*ч, but remained, also authentically, greater than in control (143% from its level). Quantitatively close to this series AST were also defined in the groups where the researchers injected APTS (1,99 + 0,07 mmol / л*ч, or 149% from control), APT (2,03 + 0,05 mmol / л*ч, 152%), APFT-1 (1,91 + 0,05 mmol / л*ч, or 143%). And only the animals who were injected with APFT-2, showed the effect (1,61 + 0,08 mmol / л*ч, or 120%) statistically authentically more evident than in other groups.

On the whole, the same regularity, but with a wider range of evidence of protective effect, is defined for the values of nuclear heating plant: if after the preventive course of RJ (1,90 + 0,11 mmol / л*ч, or 171%), APTS (1,83 + 0,12 mmol / л*ч, or 165%) and APT (1,75 + 0,08 mmol / л*ч, or 158%) and the influence of hypoxemic factors there was a moderate and equally evident protective effect, then APFT-1 (1,54 + 0,08 mmol / л*ч, or 139%) and, especially, APFT-2 (1,33 + 0,07 mmol / л*ч, or 120%) had authentically more significant (in comparison with the RJ + hypoxia series) effect. But it should be noted that in all preparation groups the hyperactivity of enzyme statistically differed from control.

The dynamics of activity of alkaline phosphatase was also characterized by the unequal changes. Here the equally evident minimal protective effect to hypoxia was revealed in cases of RJ prescription (1,98 + 0,09 mmol / л*ч, or 144% from control) and APTS (1,92 + 0,10 mmol / л*ч, or 139%), more considerable (and already authentically distinguishable from the previous) – with the animals who were injected with APT (1,71 + 0,06 mmol / л*ч, or 124%) and APFT-1 (1,59 + 0,08 mmol / л*ч, or 115%) and the effect was maximum - after APFT-2 prescription (1,41 + 0,07 mmol / л*ч, or 102%). And in two latter cases the values of enzyme activity didn't differ from the corresponding characteristics of the animals from the group of biological control.

The compared apicompositions and royal jelly had distinct and authentically close in evidence protective effect on the factors causing an acute hypoxemic hypoxia, judging by the characteristics of synthetic function of liver. Thus, the contents of the general protein in all the preparation groups appeared statistically higher than in the hypoxia series, making up 88% from control with the rats receiving RJ (73,3 + 1,9 g/l) and APTS (73,3 + 2,4 g/l), 89% (74,5 + 1,3 g/l) - APT, 93% (77,1 + 1,4 g/l) - APFT-1, 92% (76,4 + 1,9 g/l) - APFT-2. The concentration of the general cholesterol (RJ- 1,39 + 0,09 mmol/l, or 80%; APTS - 1,48 + 0,09 mmol/l, or 85%: APT - 1,55 + 0,08 mmol/l, or 89%; APFT-1 - 1,53 + 0,08 mmol/l, or 88%; APFT-2 - 1,49 + 0,11 mmol/l, or 86%) statistically significantly differed from the characteristics of the animals who were not protected from hypoxia by preventive apitherapy. And in three latter series, i.e. with APT, APFT-1 and APFT-2 injection, the contents of the general cholesterol in blood authentically didn't differ from control.

Changes in the experimental series of concentration of glucose and urea appeared more diverse. As well as in the analysis of enzymatic data, here can also be noted the "stratification" of RJ and apicompositions according to the results, allowing to single out three relative groups: a) with a moderate (but reliable in comparison with the hypoxia series) effect of RJ, APTS and APT, the injection of which promoted the preservation of concentration of these substrata within 20-30% fluctuations from a reference level; b) with a more evident effect - APFT-1: it stabilized their concentration in 90% limits and, at last, c) with the maximum protective effect - APFT-2: with this prescription the glucose and urea levels almost completely corresponded to control values.

Finally, the data of the analysis of the defined electrolytes contents in blood serum of the animals subjected to the influence of hypoxemic characteristics and against the background of injection of RJ and apicompositions with its inclusion, are the evidence of:


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firstly, the existence of the protective effect shown in all preparation series with authentically smaller extent of changes of potassium and sodium levels in comparison with the hypoxia series; and, secondly, the certain quantitative distinctions caused by this effect corresponding to the data on other biochemical indices presented above. Thus, the concentration of potassium at the animals receiving native royal jelly (4,37 + 0,111 mmol/l, or 82% from control), APTS (4,44 + 0,09 mmol/l, or 84%) and APT (4,61 + 0,17 mmol/l, or 87%), though being authentically closer to a reference level than at "purely hypoxemic" rats, significantly differed from the "APFT-1+hypoxia series" (4,88 + 0,12 mmol/l, or 92%) and the "APFT-2+hypoxia" (5,01 + 0,88 mmol/l, or 95%). The two latter groups were characterized by reliable excess of values of potassium concentration in comparison with the control series. And, essentially, the same ratios (with a little more distinction between APFT-1 and APFT-2) were drawn on sodium concentration: RJ - 130,4 + 1,2 mmol/l, or 93% from control; APTS - 129,1 + 0,8 mmol/l, or 92%; APT - 130,5 + 0,5 mmol/l, or 93%, APFT-1 - 135,0 + 1,1 mmol/l, or 96%; APFT-2 - 138,5 + 0,7 mmol/l, or 99%.

So, the comparative analysis of the complex of biochemical indices of blood serum is the evidence of existence of protective effect to the influence of hypoxemic factors both in case of royal jelly usage and apicompositions with its inclusion. In most cases this effect was close in evidence to native royal jelly in the series where there was a preventive injection of APTS and APT, and exceeded it - after the prescription of APFT-1 and, especially, APFT-2 when many of the determinated characteristics statistically didn't differ from the corresponding values of the control group of animals. The noted protective effect in relation to the chosen complex of biochemical indices can be considered as a subtler and differentiated manifestation of anti-hypoxemic effect of apicompositions containing royal jelly, in comparison with the data of gas structure and acid-base balance of blood.

Hypoxia is one of the factors initiating free radical oxidation (Vladimirov Y.A. 1987, Kolchinskya A.Z. 1993, Pozharov V.P. et al., 1993.) therefore the results are quite logical. They are the evidence of moderate extent of activization of POL with the animals who were subjected to the influence of hypoxemic factors in all analyzed tissues: hepatic (MDA - 34,01 + 2,19 nmol/g, or 239% from control; NADF-N-POL-6,18 + 0,54 nmol / г*мин, or 172%; ASK-POL - 7,11 + 0,30 nmol / г*мин, or 201%), myocardial (MDA- 51,77 + 4,22 nmol/g, or 265%; NADF-N-POL - 6,46 + 0,42 nmol / г*мин, or 218%; ASK-POL - 6,71 + 0,27 nmol / г*мин, or 189%) and brain (MDA - 27,47 + 3,01 nmol/g, or 171%; NADF-N-POL - 9,32 + 0,61 nmol / г*мин, or 140%; ASK-POL - 7,12 + 0,44 nmol / г*мин, or 127%). A more marked increase of indices of free radical oxidation took place in the myocardial tissue, though all the changes for liver and brain also appeared highly reliable.

A ten-day prescription of RJ and apicompositions before placing the animals in the hypoxemic camera was accompanied by a clear effect shown by a smaller extent of activation of lipid peroxidation. Thus, the concentration of MDA (RJ - 19,08 + 1,30 nmol/g, or 134% from control; APTS - 19,69 + 0,11 nmol/g, or 138%; APT - 20,54 + 2,27 nmol/g, or 144%; APFT-1 - 20,31 + 1,61 nmol/g, or 143%; APFT-2 - 20,69 + 2,11 nmol/g, or 145%) in liver though statistically exceeded its values in the control group, but in all cases was authentically below than in the hypoxia series. Also authentically less than only under the influence of hypoxemic factors, appeared the characteristics of activity of NADF-N-POL and ASK-POL (RJ - 4,25 + 0,33 and 4,69 + 0,21 nmol / г*мин, or 118 and 133% from control; APTS - 4,14 + 0,33 and 4,91 + 0,31 nmol / г*мин, or 115 and 139%; APT - 4,11 + 0,33 and 5,15 + 0,27 nmol / г*мин, or 114 and 146%; APFT-1 - 4,25 + 0,30 and 4,58 + 0,42 nmol / г*мин, or 118 and 129%; APFT-2 - 4,05 + 0,41 and 4,78 + 0,39 nmol / г*мин, or 113 and 135%).

Close in the directivity and extent of change of lipid peroxidation indices are marked in the preparation series and in myocardium tissue: equally evident protective effect for royal jelly and apicompositions with its inclusion was shown authentically in lower values of MDA concentrations (though higher than in control) (RJ - 25,56 + 1,71 nmol/g, or 131%; APTS - 30,30 + 4,14 nmol/g, or 155%; APT - 29,16 + 2,76 nmol/g, or 149%; APFT-1 - 28,45 + 2,93 nmol/g, or 146%; APFT-2 - 26,92 + 4,06 nmol/g, or 138%) and the speed of enzymic-dependent and nonenzymic lipid peroxidation (RJ - 4,67 + 0,24 and 3,79 + 0,28 nmol / г*мин, or 159 and 107%; APTS - 4,04 + 0,44 and 4,18 + 0,39 nmol / г*мин, or 137 and 117%; APT - 4,16 + 0,39 and 4,28 + 0,38 nmol / г*мин, or 142 and 120%; APFT-1 - 4,36 + 0,43 and 4,18 + 0,50 nmol / г*мин, or 148 and 117%; APFT-2 - 3,89 + 0,34 and 3,75 + 0,41 nmol / г*мин, или132 and 105%) than with the animals with a "pure" hypoxia.

At last, an insignificant activation of free radical oxidation in brain tissues, caused by an acute hypoxia, was almost completely prevented both by royal jelly and its combinations with other products of beekeeping.

As appears from the presented data, all the compared apicompositions in the conditions of influence of the factors leading to an acute hypoxia development had a moderate and equally evident antioxidant effect. It is not inconceivable that the decisive factor which predetermined this similarity was royal jelly as it was present at all the apicompositions and came in the rats' organisms in a standard dose of 10 mg/kg.

## CONCLUSIONS

On the whole, the received results allow concluding the following. Royal jelly and apicompositions with its inclusion, prescribed as a ten-day course, caused the increase of stability of laboratory animals' organisms

to the influence of hypoxemic factors. The anti-hypoxemic effect was shown in the minimum and close in values changes of the characteristics of gas structure and acid-base balance (in comparison with the control series). It could be connected both with overall adaptive action of injured remedies and a faster reduction of fixed characteristics after the "release" of the animals in the preparation groups in comparison with the hypoxia series. It is important to note that, judging by the results of changes of a complex of common laboratory biochemical indices of blood serum, the extent of manifestation of the analyzed action was unequal: moderate - at the prescription of native royal jelly, APTS and APT, more significant – in the series where APFT-1 was applied and maximum – with APFT-2. There was not alike concurrency for the antioxidant effect, which appeared moderate and equally evident in all the preparation series. It allows assuming the participation of other mechanisms in the realization of anti-hypoxemic action on application of the analyzed apicompositions, a different degree of activity of which can depend on propolis or pollen presence in their structure.

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