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Neurometabolic Effect of Altaian Fungus *Ganoderma lucidum* (Reishi Mushroom) in Rats Under Moderate Alcohol Consumption

Oleg B. Shevelev, Andrey E. Akulov, Anna S. Dotsenko, Galina V. Kontsevaya, Mariya A. Zolotykh, Lyudmila A. Gerlinskaya, Sergey G. Veprev, Tat'yana N. Goryachkovskaya, Natal'ya A. Zhukova, Nikolay A. Kolchanov, Sergey E. Pel'tek, and Mikhail P. Moshkin

Background: The medications produced from natural products are widely used as prophylactics for sickness induced by alcohol consumption. One such prophylactic is produced from the Reishi mushroom, *Ganoderma lucidum*. Because of the antioxidant properties of these preparations, we expect neuroprotective prophylactic effects of Reishi-based medications in alcohol-treated animals.

Methods: The Reishi (R) suspension was produced as water extract from Altaian mushrooms. Sprague–Dawley male rats were separated into the following 3 experimental groups: Group A + R received R (6 days per week) starting 1 week before alcohol exposure, and during the next 3 weeks, they received both R and alcohol; group A received alcohol; and group C received water. At the end of experiment, we determined the metabolic profile using proton magnetic resonance spectroscopy (^{1}H MRS) of the brain cortex and phosphorus magnetic resonance spectroscopy of the liver. Additionally, the blood cells were collected, and the serum biochemistry and liver histology were performed after euthanasia.

Results: Partial least squares discriminant analysis processing of the brain ^{1}H MRS gave 2 axes, the Y_1 axis positively correlated with the level of taurine and negatively correlated with the level of lactate, and the Y_2 axis positively correlated with the content of GABA and glycine and negatively correlated with the sum of the excitatory neurotransmitters, glutamate and glutamine. The Y_1 values reflecting the brain energetics for the A + R group exceeded the corresponding values for groups C and A. The maximal level of Y_2 reflecting the prevalence of inhibitory metabolites in the brain was observed in the rats exposed to alcohol. Moderate alcohol consumption did not cause significant pathological changes in the livers of the experimental animals. However, 20 days of alcohol consumption significantly increased the number of binuclear hepatocytes compared to the control. This effect was mitigated in the rats that received the Reishi extract.

Conclusions: Regular administration of the Reishi suspension improved the energy supply to the brain cortex and decreased the prevalence of inhibitory neurotransmitters that are characteristic of alcohol consumption. The alcohol-induced increase in liver proliferation was significantly suppressed by regular administration of the *G. lucidum* water suspension.

Key Words: Reishi, Alcohol, Brain Metabolites, Hepatocyte Proliferation.

THE BRAIN IS the major target organs for the negative effect of ethanol (EtOH) (Romero-Martínez and Moya-Albiol, 2013). Regular alcohol consumption leads to cognitive disorders (Cippitelli et al., 2010), neuronal dysfunctions, degenerative alterations (Crews and Nixon, 2009; Crews et al., 2000) in the hippocampus (Kelso et al., 2011; Zahr et al., 2010), brain temporal lobe (Crews and Braun, 2003; Crews and Nixon, 2009; Crews et al., 2000), and olfactory bulbs (Cippitelli et al., 2010). Developing in vivo proton magnetic resonance spectroscopy (^{1}H MRS) provided evidence of the alcohol-induced brain metabolic disturbances in human and rat (Bauer et al., 2013; Liu et al., 2014; Pennington et al., 2014; Silveri et al., 2014; Zahr et al., 2014).

Both pharmaceuticals and medications produced from natural products are used to prevent pathologies caused by alcohol consumption. In particular, such medications are produced from the fungus *Ganoderma lucidum* (Reishi mushroom, according to the name accepted in the Chinese medicine). Reishi-based medications are successfully used to treat chronic liver diseases of various etiologies. This effect of Reishi can mitigate the alcohol-induced brain disorders due to key role of liver in metabolism of xenobiotics, including alcohol (Stewart et al., 2001). It is hypothesized that the

From the Institute of Cytology and Genetics (OBS, AEA, GVK, LAG, SGV, TNG, NAK, SEP, MPM), Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia; Physiology Department (ASD, MAZ, MPM), Novosibirsk State University, Novosibirsk, Russia; Vorozhtsov Novosibirsk Institute of Organic Chemistry (NAZ, NAK), Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia; and Department of Vertebrate Zoology (MPM),Tomsk State University, Tomsk, Russia.

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*Reprint requests: Mikhail P. Moshkin, Prof, Dr.Sci.Biol., Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Prospekt Lavrentyeva 10, Novosibirsk 630090, Russia; Tel.: +7-383-363-4967*7207; Fax: +7-383-333-1278; E-mail: mmp@bionet.nsc.ru*

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G. lucidum polysaccharide and triterpenoid components protect against liver injuries caused by toxins or EtOH (Gao et al., 2003). Triterpenoids and peptides isolated from the Reishi mushroom also exhibit hepatoprotective properties against liver injuries caused by carbon tetrachloride and D-galactosamine. This is because triterpenoids display antioxidant activity and bind free radicals (Shi et al., 2008; Sun et al., 2004; Wang et al., 2002).

The antioxidant properties of Reishi mushrooms can also prevent alcohol-induced brain disorders, which develop due to oxidative stress. A Reishi polysaccharide extract efficiently inhibited the development of oxidative stress in the brain during hypoxia and reoxygenation (Zhao et al., 2004; Zhou et al., 2010).

To elucidate a neuroprotective effect of the Reishi, we studied Sprague–Dawley rats that were treated with a moderate dose of alcohol along or in combination with the same dose of alcohol and Reishi. Mushrooms, picked up in the southern region of western Siberia (Altai Mountains), were used for preparation of medications. As our study is a first attempt to test Reishi as medication for alcohol-induced changes of brain metabolism, we used suspension of mushroom containing whole composition of bioactive compounds. Because, according to Russell and Paterson (2006), the interaction between different biologically active components can be responsible for their effects in vivo.

Experiments were performed with Sprague-Dawley rats, which received Reishi suspension 1 week before alcohol exposure and during the entire experimental period (3 weeks). Among a high range of Reishi dosages, which were used for treatment of laboratory animals, we selected intragastric administration of 100 mg/kg. This dose had appreciable hepatoprotective effect in rats poisoned by CCl4 (Kwon and Kim, 2011). Dose of alcohol was chosen according to the study of Lee and colleagues (2014), which demonstrated that daily consumption of alcohol in dose 4.5 g/kg during 4 days was enough for appreciable shift of brain metabolism detected by ex vivo ^{1}H MRS. At the end of experiment, the animals were evaluated using brain cortex ^{1}H MRS to assess the metabolic changes caused by alcohol consumption whether with or without regular Reishi administration.

Also, we assessed the impacts of both alcohol consumption and combination of alcohol and Reishi on liver of studied rats. Phosphorus magnetic resonance spectroscopy (^{31}P MRS) and histology were used. As an increase in the functional intensity of detoxifying systems induces liver hypertrophy (Stewart et al., 2001), the number of binuclear hepatocytes was counted from the histological slices of liver sections as the primary indicator of lever response on the alcohol load. The general condition of the animals was estimated according to changes in their body weight, blood cell count, the blood concentrations of total protein, creatinine, cholesterol, bilirubin, and activity of aspartate aminotransferase, gamma-glutamate transferase, and acid phosphatase. The results demonstrate a preventive effect of a suspension of *G. lucidum* from Altai against moderate alcohol consumption.

MATERIALS AND METHODS

Experimental Animals and Husbandry Conditions

All animals were handled according to the regulations of the Animal Care and Use Committee of the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, and they were maintained in a pathogen-free environment. The Bioethics Review Committee of the Institute Cytology and Genetics approved the experimental protocol. The study was conducted at the Center for Genetic Resources of Laboratory Animals at the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences (RFMEFI61914X0005 and RFMEFI61914X0010). A total of 29 outbred Sprague–Dawley rats with an age of 8 to 9 weeks at the beginning of experiment were used. The animals were housed in individually ventilated cages, with 1 animal per cage. The cages had a height of 20.5 cm and an area of 929 cm^2 (OptiRAT cage; Charles River Laboratories, Chatillon-sur-Chalaronne, France). The animals were provided ad libitum water and Chara SPF granulated forage for laboratory rodents (closed company ZAO Assortiment-Agro, Puschino Russia), and they had an artificial photoperiod (14-hour light/10-hour darkness), temperature of 22 to 24°C, and humidity of 40 to 50%. Dry dedusted wood shavings (limited liability company OOO Al'bion, Novosibirsk, Russia) were used as litter. The food and litter were autoclaved before use (121°C). The drinking water was deionized with a Millipore NF-C8674 (Merck Millipore, Billerica, MA) and supplemented with Severyanka mineral additive (limited liability company OOO Eko-proect, St. Petersburg, Russia).

Experimental Medication

The mushrooms were collected in the Altai region, and all mushrooms used in the experiment were identified as *G. lucidum*. Initially, the fungi were dried to a constant weight and ground to a particle size not exceeding 150 μm (MAN-30 device; closed company ZAO MVM, Moscow, Russia). The suspension was made by shaking a weighed sample of dried mushroom with water using a vortex. The fungus concentration of the suspension was 33 mg/ml. The suspension was prepared daily immediately before administration to the animals. It was multicomponent microemulsion *G. lucidum* (He et al., 2013), which contents triterpenes, ganoderic acids, polysaccharides, lectins, and other bioactive compounds (Russell and Paterson (2006).

EtOH solution (15%) was used as the alcohol.

Experimental Scheme

All animals were divided into 3 experimental groups of approximately the same size. All administration has been carried out daily for the 6 days of each week immediately after light off (15:00 of local time).

1. *Experimental group A + R:* During 4 weeks, the animals (N = 9) intragastrically received 1-ml Reishi suspension. After first week of Reishi treatment, next 3 weeks the animals were provided water containing 15% alcohol in interval from 15:00 to 07:00 of local time. Then, bottle with 15% alcohol was replaced with water after.
2. *Experimental group A:* The animals (N = 10) were treated following the protocol of previous experimental group (A + R) with one exception—gavage of Reishi was replaced with administration of water.
3. *Control group C:* The animals (N = 10) were treated following the protocol of previous experimental group (A) with one excep-

tion—alcohol treatment was replaced with administration of water.

Every day liquid intake was measured by reduction of bottle volume for 16 hours (15:00–07:00 of local time) and for the remaining 8 hours (07:00–15:00 of local time). Also, once a week, animals were weighted.

Procedure of Gavage. The Reishi suspension and water (1 ml) were intragastrically administered to the unanesthetized rats in groups A + R, A, and C via a gastric tube. The Reishi concentration of the suspension was 33 mg/ml, corresponding to a dose of approximately 100 mg/kg for the animals with a body weight of approximately 330 g, which was tested in a study of Reishi hepatoprotective effects (Kwon and Kim, 2011).

Magnetic Resonance Spectroscopy. The rats were assayed by MRS on day 28 of experiment using a horizontal tomograph with a magnetic field intensity of 11.7 T (Biospec 117/16 USR; Bruker, Ettlingen, Germany). Two successive scanning sessions were performed. In the first session, the rat brain cortex was examined using ^{1}H radiofrequency coils. In the second session, the phosphorus profile of the liver tissue was recorded using a ^{31}P radiofrequency coil. In all cases, the rats were immobilized with a gas anesthesia (Isofluran; Baxter Healthcare Corp., Deerfield, IL) using a Univentor 400 Anesthesia Unit (Univentor, Zejtun, Malta). The animal body temperature was maintained with a water circuit installed into the table bed of the tomograph, which maintained at 30°C on its surface. A pneumatic respiration sensor (SA Instruments, Stony Brook, NY) was placed under the lower body part, which allowed for the control of the depth of anesthesia.

^{1}H-MRS. All proton spectra of the rat brain cortex were recorded with transmitter volume (500.3 MHz; distribution, 72/89 mm) and receiver surface (500.3 MHz; 123 × 64 × 31 mm) ^{1}H radiofrequency coils. High-resolution T2-weighted images of the rat brain (section thickness, 0.5 mm; field of vision, 2.5 × 2.5 mm; matrix, 256 × 256 dots) were recorded by RARE (rapid with relaxation enhancement) with the pulse sequence parameters TE = 11 ms and TR = 2.5 seconds for the correct positioning of the spectroscopic voxels (1.6 × 4.0 × 3.0 mm). Figure 1*A* shows the position of the voxel in an axial section. All proton spectra were recorded by spatially localized single-voxel STEAM (stimulated echo acquisition mode) spectroscopy with the pulse sequence parameters TE = 3 ms and TR = 5 seconds and 100 accumulations. Uniformity of the magnetic field was tuned within the selected voxel using FastMap (Bruker) before each spectroscopic recording. The water signal was inhibited with a variable pulse power and optimized relaxation delays (VAPOR) sequence.

Processing of ^{1}H Spectra. The experimental ^{1}H magnetic resonance spectra were processed, and the quantitative composition of metabolites was determined with an original specialized program similar to the LCModel software package (Provencher, 1993), assuming that the spectrum of a mixture of known compounds is a linear combination of analyzed components. See Moshkin and colleagues (2014) for a detailed description of the program operation.

^{31}P-MRS. The phosphorus spectra were recorded with a receiver–transmitter double-tuned surface ^{1}H/^{31}P radiofrequency coil (500.3/202.4 MHz; diameter, 15/20 mm). The coil was positioned to the right hypochondrium of the rat in a right lateral decubitus position, which allowed for minimization of the signal capture by muscles and a decrease of the movement artifacts. Before each session, tomography for recording 3 orthogonal packages of liver sections (Multi-slice Tri-Pilot scanning) was conducted to confirm the correct coil positioning relative to the liver tissue. The phosphorus spectra were recorded using a nonspatially localized single-pulse method with TE = 3 ms, TR = 100 ms, and 2,048 replicates. The spectrum range was 40 ppm, which made it possible to obtain information about the tissue contents of 5 substances represented by 7 peaks, phosphomonoester (PME; 6 ppm), inorganic phosphate (Pi; 5 ppm), phosphodiester (PDE; 2 ppm), creatine phosphate (PCr; 0 ppm), and adenosine triphosphate (ATP; −2.7, −7.8, and −16.5 ppm).

After tomography and spectroscopy, the animals were euthanized by decapitation and bled for assays of cell composition and biochemical analysis.

Blood Hematological Analysis. Blood hematological analysis was conducted using a Hema Screen 18 (Hospitex Diagnostics, Firenze, Italy).

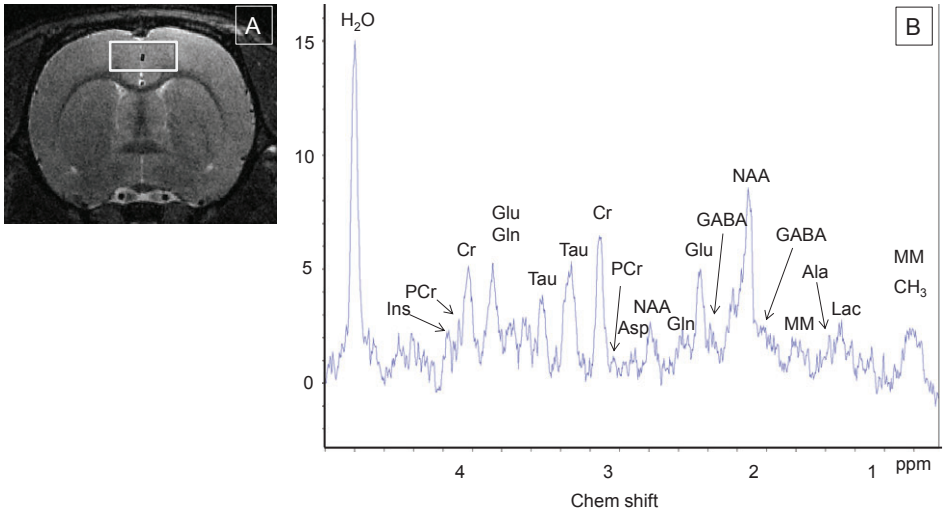


Fig. 1. (A) Position of voxel during ^{1}H MRS of the brain cortex and (B) a characteristic ^{1}H MRS spectrum. Ins, myo-inositol; PCr, phosphocreatine; Cr, creatine; Glu, glutamic acid; Gln, glutamine; Tau, taurine; Asp, aspartate; NAA, *N*-acetylaspartate; GABA, gamma-aminobutyric acid; MM, macromolecules; Ala, alanine; and Lac, lactate.

Table 1. Blood Cell Counts in Control and Experimental Rat Samples

	Leukocytes, $\times 10^9$/l	Erythrocytes, $\times 10^{12}$/l	Hemoglobin, g/l	Erythrocyte volume, fl	Platelets, $\times 10^9$/l
Control (N = 10)	11.94 ± 1.05	6.49 ± 0.13	14.66 ± 0.45	40.70 ± 2.39	550.40 ± 84.59
Alcohol (N = 10)	9.90 ± 1.02	6.67 ± 0.14	14.63 ± 0.39	37.60 ± 2.11	551.60 ± 70.06
Reishi (N = 9)	8.99 ± 0.85	6.67 ± 0.10	14.94 ± 0.40	34.44 ± 1.37	373.67 ± 27.73
$F(2, 26)$*	2.33	0.54	0.17	2.29	2.22
p*	0.12	0.63	0.85	0.12	013

*One-way ANOVA: Fisher's criteria and p-value.

Table 2. Blood Biochemical Characteristics of Control and Experimental Rats

	Total protein, g/l	Creatinine, μmol/l	Aspartate aminotransferase, AU/l	γ-Glutamyl transpeptidase, AU/l	Total bilirubin, μmol/l	Cholesterol, $\times 10$ mg/l	Acid phosphatase, AU/l
Control (N = 10)	64.62 ± 0.69	0.43 ± 0.02	141.10 ± 8.35	5.94 ± 0.28	0.78 ± 0.39	65.40 ± 2.12	5.05 ± 0.47
Alcohol (N = 10)	66.40 ± 0.79	0.44 ± 0.02	118.20 ± 5.30	6.16 ± 0.31	0.91 ± 0.17	65.50 ± 4.44	4.34 ± 0.42
Reishi (N = 9)	66.58 ± 0.92	0.40 ± 0.02	123.22 ± 5.79	6.23 ± 0.13	0.89 ± 0.27	60.22 ± 3.20	4.80 ± 0.42
$F(2, 26)$*	1.82	1.62	3.30	0.32	0.06	0.75	0.68
p*	0.18	0.22	0.055	0.73	0.94	0.48	0.51

*One-way ANOVA: Fisher's criteria and p-value.

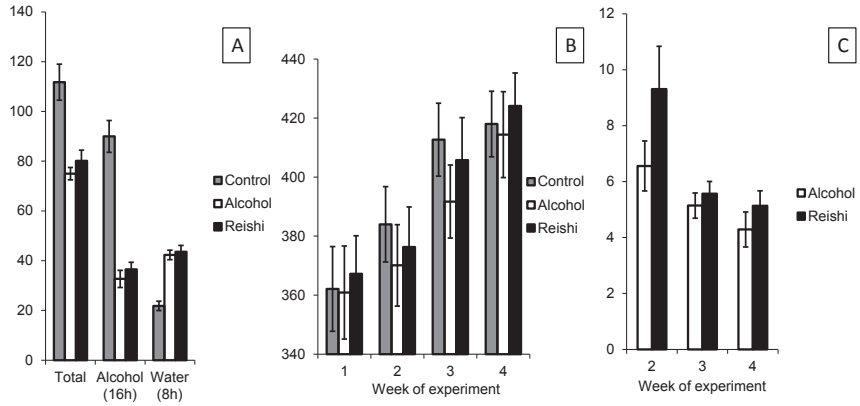


Fig. 2. Liquid intake (A), dynamic of body mass (B), and dynamic of daily alcohol consumption (C). (A) Liquid intake (ml/kg body weight) per day (total), per interval of alcohol exposure (alcohol 16 hours), per interval of water exposure (water 8 hours). (B) Dynamic of body mass (G). (C) Dynamic of daily alcohol consumption (g/kg body weight) at the 2nd, 3rd and 4th weeks of experiment.

Blood Biochemical Analysis. The blood serum was obtained by natural clotting of the blood plasma and subsequent centrifugation at 4,000×g. The supernatant was than collected and assayed using a Dimension R×L Max clinical chemistry system (Dade Behring Inc., Westwood, MA) with chip cartridges for determination of the total blood plasma protein, creatinine, aspartate aminotransferase, gamma-glutamyl transferase, acid phosphatase, bilirubin, and cholesterol (Dimension Clinical kit; Siemens, Malvern, PA).

Liver Histomorphology. The liver samples were fixed with 10% neutral formalin, embedded in paraffin, and used to make 3-μm sections. The sections were stained with hematoxylin and eosin according to a standard protocol (Lillie, 1965).

Statistical Processing. The data are shown as the mean ± SE. The mean values were compared to the control using a 1-way analysis of variance (ANOVA) with post hoc least significant difference (LSD) test. As liquid intake and body mass were measured once a week (3 to 4 times for experimental period), ANOVA with repeated measure was used for the statistical treatment of these data. The number of initial variables, characterizing the relative number of brain metabolites, was reduced with a partial least squares discriminant analysis (PLS-DA). The correlations of the PLS-DA axes with the metabolites were analyzed by Pearson's method. To pool the data for different experimental groups to analyze the correlations between the binuclear hepatocyte counts and β-ATP, the initial values were normalized using z-transformations as follows:

$$z - \text{score} = (X_{ij} - \text{Mean}_j)/\text{SD}_j,$$

where X_{ij} is the individual value of a characteristic in group J, Mean_j is the mean for the values of the characteristic in group J, and SD_j is the standard deviation in group J.

RESULTS

Liquid Intake and Body Weight

The replacement of water with 15% alcohol solution at 16 hours led to a statistically significant decrease in the daily water intake by the rats that received alcohol only and alcohol in combination with daily administration of Reishi water suspension (Fig. 2*A*). This effect was caused by a consider-

able decrease in liquid consumed at the time of alcohol treatment, during which group A differed by 2.75-fold ($p < 0.01$) from the control group and group A + R by 2.46-fold ($p < 0.01$). Daily alcohol consumption calculated from 15% solution and body mass of animals progressively declined from 6.7 ± 0.7 g/kg (group A) and 8.7 ± 1.3 g/kg (group A + R) at the first week to 4.3 ± 0.6 g/kg (group A) and 5.1 ± 0.5 g/kg (group A + R) at the last week (Fig. 2*C*). ANOVA with repeated measures revealed statistically significant effect of repetition, $F(2, 34) = 19.03$, $p < 0.001$, and insignificant effect of group, $F(1, 17) = 1.80$, $p < 0.19$.

During the time of water supplying, the liquid intake by the rats of both experimental groups increased (Fig. 2), but did not completely compensate diurnal consumption. Despite the different liquid intakes, the body weight increased almost equally for the control and experimental rats (Fig. 2*B*). The effect of experimental group was statistically insignificant, $F(2, 24) = 0.05$, $p = 0.95$; ANOVA with repeated measure, whereas the effect of the repeated measure was statistically significant, $F(3, 72) = 149.5$, $p < 0.001$.

Blood Parameters

One-way ANOVA did not show any statistically significant effect of alcohol consumption (group A) and combination of alcohol and Reishi (group A + R) on the blood cells, concentrations of hemoglobin, creatinine, bilirubin, cholesterol, and activity of the enzymes (Tables 1 and 2).

Table 3. Levels of Brain Cortex Metabolites in Control and Experimental Rats (In Vivo MRS)

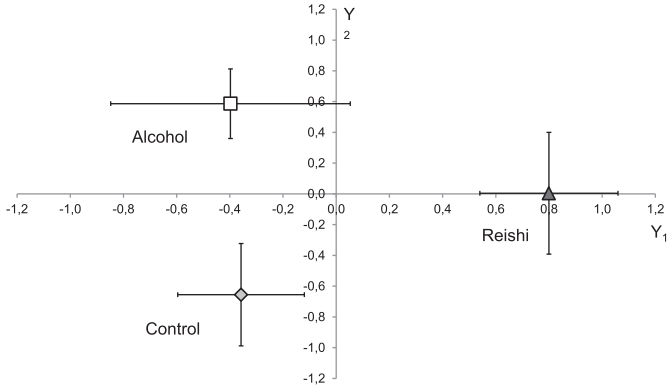
	NAA	GABA	Ala	Ast	Cho	Cr + PCr
Control (*N* = 9*)	18.3 ± 0.4	5.1 ± 0.7	3.6 ± 1.0	0.82 ± 0.45	1.63 ± 0.22	14.3 ± 0.6
Alcohol (*N* = 10)	19.2 ± 0.7	6.3 ± 0.4	4.3 ± 1.2	0.26 ± 0.10	1.52 ± 0.25	13.4 ± 0.8
Reishi (*N* = 9)	18.9 ± 0.6	5.9 ± 1.0	6.2 ± 1.1	0.60 ± 0.27	1.18 ± 0.23	13.2 ± 0.8
F(2, 26)**	0.55	0.63	1.37	0.93	0.98	0.54
*p***	0.58	0.54	0.27	0.40	0.39	0.59

	Glu + Gln	mIno	Tau	Gly	Lac	PEA
Control	19.2 ± 1.9	2.7 ± 1.0	7.0 ± 0.3	22.1 ± 3.5	6.4 ± 1.5	10.8 ± 3.3
Alcohol	19.2 ± 1.2	4.1 ± 1.8	6.7 ± 0.4	25.6 ± 1.8	8.4 ± 1.8	12.2 ± 2.1
Reishi	19.7 ± 2.0	3.9 ± 1.5	7.3 ± 0.3	19.9 ± 1.2	5.4 ± 1.2	12.7 ± 2.6
F(2, 26)**	0.03	0.26	0.75	0.68	0.97	0.13
*p***	0.97	0.77	0.48	0.51	0.39	0.88

NAA, N-acetylaspartate; GABA, gamma-aminobutyric acid; Ala, alanine; Ast, aspartate; Cho, choline; Cr, creatine; PCr, phosphocreatine; Glu, glutamic acid; Gln, glutamine; mIno, myo-inositol; Tau, taurine; Gly, glycine; Lac, lactate; PEA, phosphoryl ethanolamine.

*One case was excluded due to technical error during MRS.

**One-way ANOVA: Fisher's criteria and *p*-value.



Y1	Control	Alcohol	Reishi	Y2	Control	Alcohol	Reishi
Control		0,934	**0,027**	Control		**0,010**	0,164
Alcohol	0,934		**0,019**	Alcohol	**0,010**		0,206
Reishi	**0,027**	**0,019**		Reishi	0,164	0,206	

Fig. 3. Distribution of experimental animal groups in the space of the Y_1 and Y_2 axes obtained by analyzing the ratio of metabolites by partial least square discriminant analysis. The data are shown as the mean ± SE. The impact of experimental group was statistically significant for both axes (1-way ANOVA)—$F(2, 25) = 3.90$, $p = 0.033$ for Y_1 and $F(2, 25) = 3.83$, $p = 0.035$ for Y_2. The *p*-values for the between-group comparisons (post hoc LSD test) are shown in the tables.

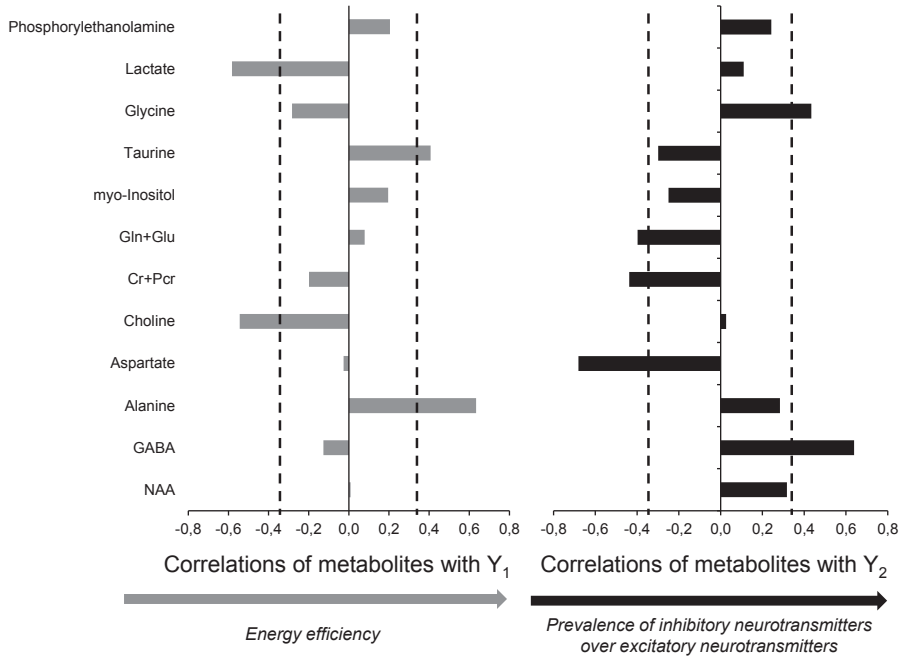


Fig. 4. Correlations of individual metabolites with the integral characteristics of metabolic patterns (the values of the Y_1 and Y_2 axes according to the partial least squares discriminant analysis). The dashed line shows the statistical significance boundary for the correlation coefficient ($p < 0.05$).

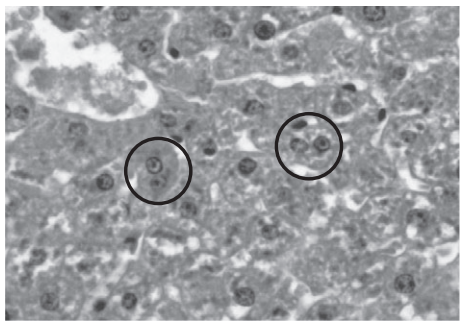


Fig. 5. Liver histological section. The circles indicate binuclear hepatocytes.

Table 4. Binuclear Hepatocyte Counts in Control and Experimental Rats

	Binuclear hepatocytes, per 2,000 cells
Control ($N = 10$)	34.1 ± 2.3
Alcohol ($N = 10$)	77.8 ± 4.0
Reishi ($N = 9$)	53.6 ± 3.6
$F(2, 26)$*	44.29
p*	<0.001

*One-way ANOVA: Fisher's criteria and p-value.

^1H MRS of the Brain Cortex

Proton MRS of the brain cortex (Fig. 1) showed that the concentrations of individual metabolites in the different rat groups were not statistically significant (Table 3). The between-group differences in the metabolomics patterns were detected using multivariate statistical analysis, which is standard for MRS data (Allen et al., 2013). The PLS-DA showed 2 axes characterizing the variation of statistically linked variables (Fig. 3). Because the values of the Y_1 axis are positively correlated with the level of a nootropic metabolite, taurine, and are negatively correlated with the level of lactate, the variations in this axis may reflect the variations in the efficiency of brain energy metabolism (Fig. 4). The Y_1 values in group A + R exceeded the corresponding values for groups C and A (Fig. 3). For the Y_2 axis, positive correlations were detected for the concentrations of the inhibitory neurotransmitters GABA and glycine and negative correlations were observed with the sum of the excitatory neurotransmitters glutamate and glutamine (Fig. 4). The maximal values of this axis, reflecting the prevalence of inhibitory metabolites in the brain, were observed in the rats exposed to alcohol (Fig. 3), with an intermediate position for group A + R.

Hepatocyte Proliferative Activity and ^{31}P MRS of Liver

Short-term alcohol consumption at a moderate dose did not cause any significant liver injuries. Although it was manifested that statistically significant increase of the binuclear hepatocyte counts is an indicator of the proliferation response on alcohol load (Fig. 5). The groups were as follows according to the binuclear cell counts per 2,000 analyzed hepatocytes: A > A + R > C (Table 4).

Analysis of the phosphorus spectra (Fig. 6) showed no statistically significant differences in the concentrations of high-energy compounds in the livers of the control and experimental rat groups (Table 5). Notably, all groups displayed a negative correlation between the ATP level in the liver and the binuclear hepatocyte counts (Fig. 7). The correlation coefficient for the overall sample with leveled

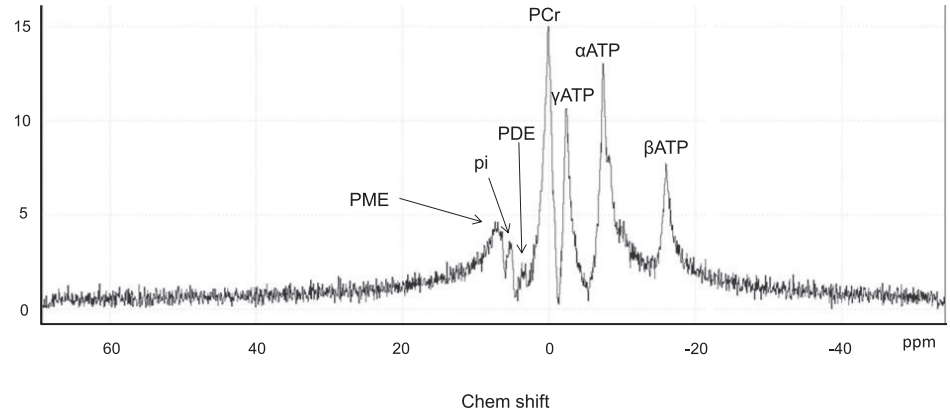


Fig. 6. A typical ^{31}P MRS spectrum of the liver. PME, phosphomonoester; pi, phosphate; PDE, phosphodiester; PCr, phosphocreatine; and γ, β, αATP, γ-, β-, and α-adenosine triphosphates.

Table 5. Levels of Liver Metabolites (In Vivo MRS)

	ATP-γ	ATP-α	ATP-β	Phosphodiester	Pi	Phosphomonoester	Anabolic charge	PCr/ATP-β
Control (N = 10)	0.72 ± 0.03	1.09 ± 0.06	0.63 ± 0.04	0.12 ± 0.01	0.19 ± 0.01	0.34 ± 0.02	0.74 ± 0.01	1.62 ± 0.09
Alcohol (N = 10)	0.69 ± 0.04	1.07 ± 0.05	0.58 ± 0.03	0.11 ± 0.02	0.18 ± 0.02	0.33 ± 0.03	0.76 ± 0.02	1.75 ± 0.10
Reishi (N = 9)	0.63 ± 0.04	0.98 ± 0.06	0.54 ± 0.04	0.10 ± 0.01	0.17 ± 0.01	0.28 ± 0.03	0.74 ± 0.02	1.93 ± 0.13
$F(2, 26)$*	1.46	1.09	1.72	1.00	0.40	1.28	0.33	1.95
p*	0.25	0.35	0.20	0.38	0.67	0.30	0.72	0.16

*One-way ANOVA: Fisher's criteria and p-value.

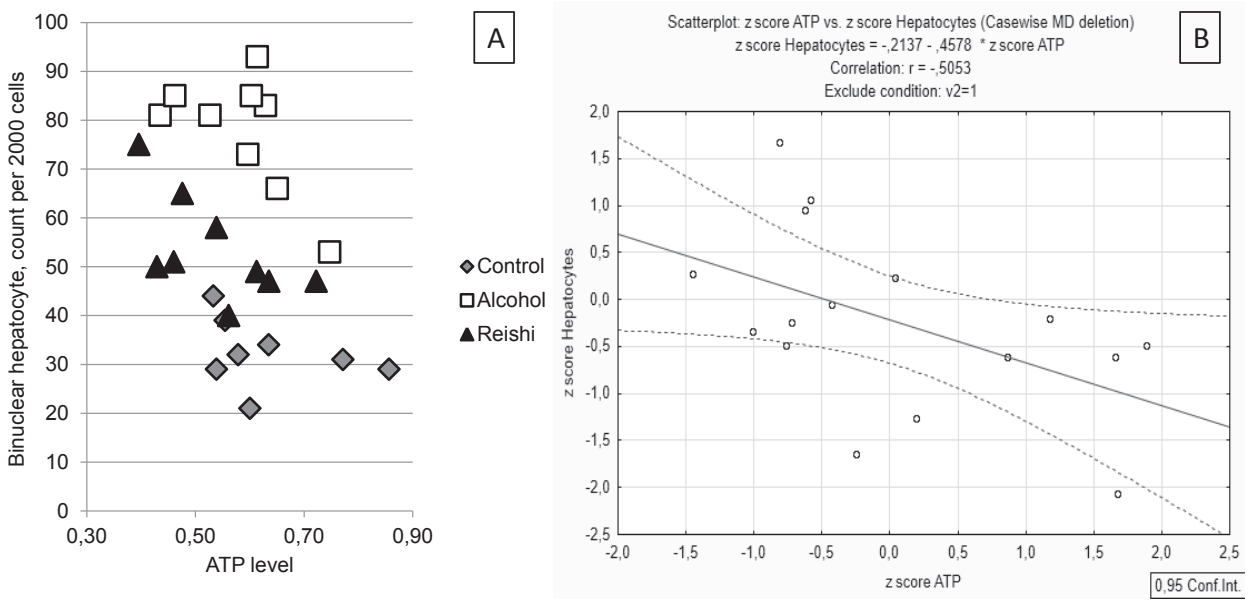


Fig. 7. Correlation of the β-ATP level with the binuclear hepatocyte counts in (A) individual experimental groups and (B) in a pooled sample after normalization of the individual values (z-score) in the individual experimental groups.

between-group differences for both parameters was −0.52 ($p < 0.01$).

DISCUSSION

At the end of experiment, the alcohol consumption in both experimental groups was very similar (4.3 ± 0.6 g/kg per day for group A and 5.1 ± 0.5 g/kg per day for group A + R) to the low dose of alcohol that was used in study Lee and colleagues (2014). In our study, alcohol exposure caused a decrease in the daily water intake because of the significant reduction in the liquid intake, when the water was replaced with a 15% EtOH solution. However, control and both experimental groups, A and A + R, dis-

played similar body weight changes. The body weight of experimental animals does not differ from the control even when the daily alcohol intake is higher (Alele et al., 2013; Sullivan et al., 2006).

Consumption of the alcohol solution itself and in combination with administration of Reishi had no effect on the studied blood characteristics. The daily alcohol dose and duration of intake did not cause any significant liver injuries. For the regular alcohol consumption in rats of group A, a significant increase in binuclear hepatocyte counts was observed. The increase in ploidy is determined by an increase in proliferative activity, which is a primary organ response to an elevated functional load (Gentric et al., 2012). A further increase in the dose may lead to clinical liver injuries and eventually, hepatocyte malignancy (Gentric et al., 2012). Administration of the Reishi suspension significantly decreased the alcohol-induced proliferative response of the liver.

The increase in cell ploidy in the organs caused by an elevated functional load is combined with changes in energy metabolism, in particular, a decrease in the level of accessible ATP (Meerson, 1986). The ^{31}P MRS of the liver showed only a trend toward decreased ATP in the group A and A + R rats compared to the control. By contrast, the binuclear hepatocyte counts, reflecting the proliferative activity, were the highest in individuals with a minimal ATP level.

In general, the body weight dynamics, blood cell counts and biochemical characteristics, and liver histology and ^{31}P MRS suggest that the general state of the experimental animals did not deteriorate. At the same time, ^{1}H MRS revealed appreciable shift of brain metabolism in alcohol-treated rats. Integrated PLS-DA of the entire set of detected chemical compounds showed statistically significant between-group differences in the metabolome patterns. The first PLS-DA axis (Y_1), containing lactate and choline with a negative correlation, and alanine and taurine with a positive correlation, were maximal in rat group A + R. This suggests an increase in the efficiency of brain energy metabolism with regular Reishi administration compared to the control group and the group that received alcohol alone. The between-group comparisons for the second PLS-DA axis (Y_2), in which the major contributors are GABA and glycine with a positive correlation, and glutamine + glutamate with a negative correlation, demonstrate that the balance between the inhibitory and excitatory neurotransmitters in group A rats is shifted toward inhibitory metabolites.

Shift of the balance between the inhibitory and excitatory neurotransmitters in alcohol-treated rats is in line with some clinical and experimental observations. ^{1}H MRS of cortex revealed higher concentration of GABA and less concentration of glutamate in heavy drinking patients with post traumatic stress disorder in comparison with nondrinking patients with post traumatic stress disorder (Pennington et al., 2014). Wistar rats, which have received a low dose of alcohol (4.5 g/kg per day) during 4 days, showed statistically significant increase of the concentration of GABA in the frontal cortex (Lee et al., 2014). Nonetheless, in alcohol-dependent patients opposite changes of the inhibitory/excitatory neurotransmitter balance in the anterior cingulate cortex were described (Bauer et al., 2013). The contradictory findings of the present data could be related with differences in dose (Lee et al., 2014), alcohol exposition time, and specificity of the alcohol-induced psychic disorder.

According to our data, the alcohol-induced imbalance of the inhibitory/excitatory neurotransmitter could be mitigated by means regular administration of the Reishi suspension, which also improved the energy supply to the brain cortex. There is the first experimental evidence of the preventive efficiency of medications produced from the Altaian mushrooms (*G. lucidum*) in experimental simulation of potential brain disorder due to regular consumption of the moderate dose of alcohol.

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