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Possibilities of Using Phyto-Preparations to Increase the Adaptive Capabilities of the Organism of Test Animals in Swimming

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Abstract: Background: To study the possibilities of using phytopreparations to increase the adaptive capabilities of the animals on which the experiments were conducted in swimming. Methods: 100 mongrel male rats were divided into 5 groups of 20 animals in each one. For 30 days running, the animals were immersed for 10 min in a bath with water at a temperature of +4 °C. In addition to cold exposure, the animals of the first three groups were injected per os with stress protectors 30 min before the immersion in water. The rats of the first group received an inhibitor of the enzyme gamma-butyrobetaine hydroxylase, the second group was given an extract of Eleutherococcus, and the third group took an extract of Ligusticum wallichii. As a placebo, to control the effect of the stress protectors, the rats of the fourth group were injected per os with 0.9% NaCl solution, and the animals in the fifth group were not given any drugs. On days 1, 4 and 30 of the experiment, five randomly selected animals from each group were decapitated, the heart and liver were removed, and the activity of tissue enzymes—superoxide dismutase (SOD), glutathione peroxidase (GPO), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)—was analyzed. Results: The animals in the control group displayed a decrease in the activity of most of the studied enzymes, increasing from the 1st to the 30th day of the experiment. The NaCl solution had practically no effect on the analyzed parameters. Against the use of the enzyme gamma-butyrobetaine hydroxylase inhibitor, the activity of the enzymes did not change as compared with the pre-intervention level. On the first day of ingestion, the effects of the Ligusticum wallichii extract were similar to those of the enzyme gamma-butyrobetaine hydroxylase inhibitor. On the 30th day of ingestion, the effects of the Eleutherococcus extract were practically indistinguishable from those of the enzyme gamma-butyrobetaine hydroxylase inhibitor. Conclusions: The data obtained suggest the presence of cytoprotective effects in the two phytopreparations that are similar to the enzyme gamma-butyrobetaine hydroxylase inhibitor. In this case, the effect of the extract of Ligusticum wallichii is more pronounced under the acute stress conditions, and the extract of Eleutherococcus, under the chronic stress conditions.

Keywords: stress; swimming; rats; phytopreparations

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1. Introduction

The results of numerous researches demonstrate that cold water stimulates the tissue respiration processes [047001 4–4]. Several factors can determine differences between body organs, including oxygen consumption, susceptibility to oxidants and antioxidant enzyme activation, antioxidant levels, and other repair systems. The response of the muscles and the heart to oxidative stress appears to be quite different than that of other body organs, such as the brain and liver. This is likely to be due to the difference in mitochondrial biogenesis and the occurrence of oxidant-induced degeneration [1].

Immersion and submersion, which are the two elements of drowning, interact with basic physiological factors such as temperature and oxygen. However, there is little scientific evidence on these mechanisms in the extreme or lethal circumstances resulting from drowning, while few studies have reported how they interact (directly or indirectly), and how they are influenced by autonomous protective (diving response, breath-holding, acute hypothermia) and life-threatening (cold shock, autonomic conflict, aspiration) responses. The theories that explain how drowning is affected by these mechanisms have remained unchanged for decades. Based on current understanding and knowledge, it is suggested that little is known about the pathophysiological events that are associated with drowning [2].

Knowledge of the work performed by swimming animals is critical to the interpretation of the importance of the research on how drugs influence swimming performance. Many studies in which forced swimming was used, have failed to consider, in detail, the complex physiological readjustments occurring when animals were forced to work in water with various temperatures. The knowledge that swimming in neutral water represents a metabolic cost that is three-times higher than basal for some rats, does not entirely reflect the physiological adjustment. When swimming in water at a temperature of 37 °C, the water must be accompanied by a shift in the volume of blood circulating to the periphery, and concurrent changes in cardiovascular performance. The factors that lead to exhaustion of the animal swimming in cold or hot water may not be associated with muscular fatigue [3].

According to some studies, the exposure to cold water on the skin of athletes can increase their performance by stimulating the processes of lipolysis [4–6].

The changes in the antioxidant defense system are tissue-specific. However, chronic exposure to cold leads to oxidative stress, by changing the prooxidant–antioxidant balance of this defense system, through increasing the prooxidants while depleting the antioxidant capacities [4].

Furthermore, the changes in the analyzed components of the antioxidant defense system, caused by prolonged exposure to low temperatures, suggest that reorganization of its activity occurs at the molecular level. Although some studies have indicated that a 21-d cold exposure was sufficient to induce adaptation of thermogenesis, a study demonstrated that, in general, longer periods are required for recording changes in the antioxidant defense system [5].

It was found that the exposure to the extended (48 h) cold (8 °C) led to alterations in both the antioxidant defense system (tissue and enzyme-specific) and serum lipoprotein profiles in rats [6].

The permanent exposure of temperature leads to certain metabolic changes caused by hormonal deviations. Cold stimulation under conditions of substantially lower temperatures induced cardiac damage, which was further aggravated by ACS; however, cold stimulation at only 3 °C lower than normal temperature improved the adaptability of broilers to ACS [7,8].

According to some scientific studies, the cold stress effects may influence the cytolysis processes of muscle cells, by stimulating them [9,10]. It concerns, first of all, cardiomyocytes. Some research works say that these changes occur due to the reduction in antioxidant enzymes activity [11,12]. Some of the authors declare that the negative effects of cold stress can be reduced by using some adaptogens. The latter was tested in athletes, with further analysis, but has never been explored in test animals.

Physical exercise in water with a low temperature was found to lead to changes in the oxidant/antioxidant balance, as a result of the repeated exposure to nondamaging mild stress factors, with greater effects of exposure compared to the physical exercise of the same intensity, but performed in thermal comfort. Furthermore, the results obtained lead to the conclusion that there are sex-dependent differences in the aging effects on lipid peroxidation. It appears that female rats may be better adapted to the changes in ambient temperature, by developing morphological, but also antioxidant, defense mechanisms, such as increased erythrocyte SOD activity and GSH concentration, in order to restore the pro-oxidant/oxidant balance. It was also found that, compared to females, aging male rats had a reduced capacity to increase their metabolic response to low temperatures. It was suggested that exposure to low temperatures is a stressor that causes the increased pro-oxidant activity of the body, and, after taking into account an appropriate exposure time, it can induce an adaptive response, which is especially noticeable in female rats [13].

In recent years, there has been an increasing interest in the use of herbal medicines in sports medicine. The effect of plants is explained by their biologically active compounds, such as polyphenols, terpenoids, and alkaloids, which have a number of physiological effects on the body, including adaptogenicity [14,15]. The positive effect of phytopreparations on immunity is shown in [16,17]. Under the influence of phytopreparations, oxidative stress decreases [18]. Some herbs have an analgesic effect [19].

The most commonly used phytopreparations are Ligusticum wallichii [20,21], Eleutherococcus [15,22], and others [23,24]. These drugs are widely used in traditional Chinese and Russian medicine. The protective properties of Ligusticum wallichii in various models are described [25–29].

There were publications about adaptogenic effects in Eleutherococcus [29–32]. There are publications that meldonium is a stress protector, especially in cold swimming conditions [33,34]. At the same time, the research results are very contradictory. Some authors call meldonium the "gold standard" for stimulating physical performance in swimming [32,35]. Based on the above, the purpose of our work was to study the phytopreparations that are potential to increase the adaptive capabilities of the body of experimental animals during swimming.

2. Materials and Methods

In the experiment, 100 mongrel male rats weighing 350–400 g were used, kept on a standard vivarium diet. The animals were kept in a suitably equipped vivarium with sufficient lighting and ventilation in special plastic cages locked with a metal mesh. Animals were fed dry food and drink ad libitum. During the experiment, the weight of the animals did not change.

To simulate cold water immersion, rats were immersed in a bath with cold (t = +4 °C) water daily. The amount of water was such that the animals could not reach the bottom. The animals were forced to swim for 10 min, after which the animals were taken out, water being removed from their hair, and placed back in the cage. The animals were immersed in cold water once a day throughout 30 days.

The animals were randomly subdivided into 5 groups of 20 rats each. In addition to cold exposure, the animals of the first 3 groups were injected with stress protectors 30 min before immersion in cold water. The animals of the first group (n = 20) were injected per os 0.15 mL of 0.5% solution of the enzyme gamma-butyrobetaine hydroxylase inhibitor (meldonium) (Nizhpharm, Russia, Nizhny Novgorod), which was brought to a volume of 5 mL with 0.9% NaCl solution. The animals of the second group (n = 20) were injected per os with solution of the phytopreparation Eleutherococcus in a dose of 5 mL (Vifitech ZAO, Russia, Moscow region). The animals of the third group (n = 20) were injected per os Ligusticum wallichii extract (Sunrider®, USA, Torrance) at a dose of 5 mL. As a placebo to control the effect of stress protectors, rats of the fourth group (n = 20) were injected per os with 0.9% NaCl solution at a dose of 5 mL. Animals of the fifth group (n = 20) were not injected with drugs (control).

Immediately after cold exposure, 5 randomly selected rats from each group were decapitated under ether anesthesia, in compliance with the rules of euthanasia, according to Helsinki Declaration on the humane treatment of animals. The animals were slaughtered on the 1st, 4th and 30th days of the experiment, which corresponds to acute, subacute and chronic stress effects. We slaughtered the animals after the cold exposure. Also, 5 randomly selected animals from each group were slaughtered before cold exposure to determine the initial activity of the enzymes (Table 1). There were no differences in the weight of the organs isolated from the animals in different groups.

Table 1. Initial activity of enzymes in groups.

	SOD (μmol/min g)		GPO (mmol/min g)		AST (mmol/min g)		ALT (mmol/min g)	
	Heart	**Liver**	**Heart**	**Liver**	**Heart**	**Liver**	**Heart**	**Liver**
Control group	49.2 ± 8.6	337 ± 149	432 ± 131	161 ± 112	91.3 ± 36.8	115 ± 17.6	129 ± 83.8	117 ± 51.0
0.9% NaCl solution	43.5 ± 23.7	347 ± 103	419 ± 109	191 ± 132	105 ± 58.0	119 ± 90.2	162 ± 92	94.5 ± 56.3
Inhibitor of the enzyme gamma-butyrobetaine hydroxylase	50 ± 17	321 ± 134	450 ± 93	217 ± 107.9	109 ± 52	105 ± 76	154 ± 117	107 ± 56
Ligusticum wallichii extract	45.3 ± 16.4	350 ± 126	405 ± 101	232 ± 103	106 ± 47.0	103 ± 56.3	122 ± 93	98.3 ± 27.9
Eleutherococcus extract	23.4 ± 5.7	385 ± 77.3	453 ± 86.7	241.5 ± 81.2	105 ± 63	113 ± 96	129 ± 91.9	112 ± 98

Note: in the table, the data is presented as: average ± standard deviation.

We confirm that all methods were carried out in accordance with relevant guidelines and regulations. We confirm that all experimental protocols were approved by the interuniversity ethics committee of the A.I Evdokimov Moscow State University (protocol No. 04-17 dated 20 April 2017). The authors followed the humane treatment of animals in accordance with the ARRIVE guidelines. All animal studies were conducted in full conformity with the international guiding principles for biomedical research involving animals (Geneva, 1990). Anesthesia was used to euthanize the animals after the tests.

The heart and liver were removed from the animals, a 0.2 g sample was obtained, then tissues were crushed and homogenized in a porcelain mortar in cold conditions, with the addition of 2 mL of a 0.5% solution of Tris–HCl buffer (pH = 7.3) and quartz sand. The tissue homogenates were centrifuged for 15 min at 3000 rpm. In the resulting supernatant, the activity of the following antioxidant enzymes was determined: superoxide dismutase (SOD) and glutathione peroxidase (GPO) (set "Randox", UK, Kearneysville), as well as the following intracellular enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (set of reagents CJSC "Vector", Russia, Koltsovo, Novosibirsk). The activity of enzymes AST, ALT, GPO was expressed in mmol/min g of tissue, and the activity of SOD was expressed in μmol/min g of tissue. The change in the enzyme activity related to their initial level (before the onset of stress exposure) was calculated as a percentage.

Statistical processing of the obtained data was carried out in such programs as Excel 2007 and Statistica 13. Fisher's method was used to compare the values expressed as a percentage. Confidence intervals were determined on basis of calculating odds ratios (OR) with $p < 0.05$.

3. Results

Under the acute stress conditions (Table 2) of the animals in the control group, there was a decrease in the activity of SOD, GPO, AST and AST of the heart. The activity of liver AST increased, and liver ALT decreased. In the animals treated with saline, in general, similar changes were observed. With the use of the enzyme gamma-butyrobetaine hydroxylase inhibitor, the activity of the enzymes did not change as compared to the intact

level; the activity of liver AST became significantly lower than in the animals of the control group, and the activity of heart AST, and heart and liver ALT was significantly higher. Against the background of Ligusticum wallichii use, the changes were similar; however, the activity of liver GPO and heart AST significantly increased as compared to the initial level. In the group of animals that were treated with Eleutherococcus, compared with the initial level, the activity of SOD of the heart increased, and the activity of SOD of the liver, AST of the liver and ALT of the liver decreased. Changes in the activities of SOD of the heart, AST of the heart, AST of the liver, ALT of the liver were multidirectional (each drug caused its own changes) in the rats of the control group and the rats treated with Eleutherococcus.

Table 2. Changes in enzyme activity from baseline under acute stress conditions.

Group	Group Number	SOD		GPO		AST		ALT	
		Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver
Control group	1	71.43% (61.33% ÷ 91.23%) *	90.62% (84.10% ÷ 103.40%)	73.61% (63.76% ÷ 92.93%) *	84.62% (76.55% ÷ 100.43%)	53.85% (42.70% ÷ 75.69%) *	380.87% (307.73% ÷ 524.21%) *	37.21% (26.40% ÷ 58.39%) *	6.45% (0.96% ÷ 17.22%) *
0.9% NaCl solution	2	74.70% (64.98% ÷ 93.75%) *	61.74% (50.88% ÷ 83.05%) *,1	74.36% (64.60% ÷ 93.50%) *	87.43% (80.02% ÷ 101.96%)	77.14% (67.75% ÷ 95.55%)	252.07% (208.29% ÷ 337.88%) *	19.89% (10.96% ÷ 37.38%) *	30.85% (20.52% ÷ 51.09%) *,1
Inhibitor of the enzyme gamma-butyrobetaine hydroxylase	3	96.00% (91.62% ÷ 104.59%) 1	92.83% (87.07% ÷ 104.14%) 2	85.60% (77.75% ÷ 100.99%)	112.90% (104.37% ÷ 129.63%)	105.16% (99.95% ÷ 115.37%) 1,2	112.42% (104.07% ÷ 128.80%) 1,2	108.27% (101.58% ÷ 121.38%) 1,2	112.74% (104.26% ÷ 129.35%) 1,2
Wallich Ligusticum extract	4	111.11% (103.25% ÷ 126.51%) 1,2	92.00% (85.93% ÷ 103.89%) 2	105.54% (100.14% ÷ 116.15%) 1,2	225.86% (188.16% ÷ 299.76%) *1–3	272.50% (224.02% ÷ 367.52%) *1–3	110.34% (102.79% ÷ 125.15%) 1,2	105.47% (100.10% ÷ 116.01%) 1,2	121.43% (110.02% ÷ 143.78%) 1,2
Eleutherococcus extract	5	134.78% (119.47% ÷ 164.79%) *,1–3	48.65% (37.47% ÷ 70.55%) *,1,3,4	81.05% (72.28% ÷ 98.22%) 4	78.05% (68.79% ÷ 96.19%) 3,4	94.65% (89.62% ÷ 104.51%) 1,4	25.08% (15.39% ÷ 44.08%) *,1–4	9.69% (3.07% ÷ 22.65%) *,1,3,4	91.99% (85.92% ÷ 103.89%) 1,2,4

Notes. Here and below $p < 0.05$; *—differences from the initial level of enzyme activity; 1–4—differences from the level of enzyme activity in animals of the corresponding group; results present as odds ratios with $p < 0.05$ interval.

Under subacute stress conditions (Table 3) in the animals of the control group, the activity of SOD and GPO decreased both in the heart and the liver. In this case, the activities of AST and ALT in both the organs increased; however, the increase in AST activity occurred to a greater extent. The introduction of a saline solution practically did not change these parameters, in comparison with the animals of the control group. When the enzyme gamma-butyrobetaine hydroxylase inhibitor was administered, the activities of all the enzymes, except for liver GPO, were indistinguishable from the intact (pre-intervention) level. In the animals of this group, the activities of all the enzymes, except for liver ALT, significantly differed from the rats of the control group: SOD and GPO upward, and heart AST and ALT downward. The effects of the Ligusticum wallichii extract were generally indistinguishable from the enzyme gamma-butyrobetaine hydroxylase inhibitor, except for the activities of SOD and ALT of the heart, which were lower against the background of a phytopreparation, compared with a synthetic inhibitor. In the group of animals that received the Eleutherococcus extracts, an increase in the activity of SOD of the heart was observed, in comparison with the rats that were given the enzyme gamma-butyrobetaine hydroxylase inhibitor. The effects of two phytopreparations differed in their influence on the activity of SOD, GPO and AST of the heart.

Table 3. Changes in enzyme activity from baseline under conditions of subacute stress.

Group	Group Number	SOD		GPO		AST		ALT	
		Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver
Control group	1	69.39% (59.08% ÷ 89.59%) *	36.16% (25.41% ÷ 57.21%) *	19.91% (10.98% ÷ 37.41%) *	41.24% (30.24% ÷ 62.82%) *	558.24% (445.15% ÷ 779.91%) *	183.48% (155.80% ÷ 237.72%) *	485.27% (388.59% ÷ 674.77%) *	127.19% (114.04% ÷ 152.96%) *
0.9% NaCl solution	2	44.58% (33.46% ÷ 66.36%) *	43.85% (32.75% ÷ 65.59%) *	32.36% (21.90% ÷ 52.86%) *	43.98% (32.88% ÷ 65.73%) *	407.62% (328.44% ÷ 562.81%) *	198.82% (167.47% ÷ 260.25%) *	424.31% (341.36% ÷ 586.89%) *	165.96% (142.56% ÷ 211.81%) *
Inhibitor of the enzyme gamma-butyrobetaine hydroxylase	3	110.00% (102.58% ÷ 124.54%) 1,2	97.20% (93.50% ÷ 104.43%) 1,2	84.00% (75.80% ÷ 100.07%) 1,2	117.97% (107.68% ÷ 138.15%) *1,2	105.46% (100.10% ÷ 115.98%) 1,2	87.58% (80.20% ÷ 102.03%) 1,2	96.46% (92.32% ÷ 104.56%) 1,2	111.46% (103.47% ÷ 127.13%) 2
Ligusticum wallichii extract	4	86.67% (79.07% ÷ 101.56%) 2,3	82.86% (74.43% ÷ 99.37%) 1,2	92.67% (86.85% ÷ 104.09%) 1,2	125.43% (112.80% ÷ 150.18%) *1,2	95.00% (90.13% ÷ 104.55%) 1,2	82.76% (74.31% ÷ 99.31%) 1,2	61.68% (50.81% ÷ 82.99%) *,1–3	111.37% (103.41% ÷ 126.97%) 2
Eleutherococcus extract	5	147.83% (129.02% ÷ 184.68%) *,1–4	90.27% (83.64% ÷ 103.26%) 1,2	65.36% (54.72% ÷ 86.21%) *,1,2,4	114.63% (105.48% ÷ 132.58%) *,1,2	92.08% (86.04% ÷ 103.92%) 1,2	87.46% (80.05% ÷ 101.97%) 1,2	95.16% (90.35% 104.57%) 1,2,4	95,92% (91,49% ÷ 104.59%) 1,2

Notes. Here and below $p < 0.05$; *—differences from the initial level of enzyme activity; 1–4—differences from the level of enzyme activity in animals of the corresponding group; results present as odds ratios with $p < 0.05$ interval.

In chronic stress conditions (Table 4), one can speak of a violation of the processes of antioxidant cells protection against the background of increasing processes of cytolysis. In this case, the inhibitor of the enzyme gamma-butyrobetaine hydroxylase, as in the case of acute and subacute stress, has a protective effect. Under chronic stress conditions, the effects of the Eleutherococcus extract are more similar to those of the enzyme gamma-butyrobetaine hydroxylase inhibitor than the Ligusticum wallichii extract. Apparently, Ligusticum wallichii extract is more cytoprotective against hepatocytes than cardiomyocytes, as evidenced by an increase in the AST/ALT ratio of the heart against the background of the decrease in this ratio for the liver.

Table 4. Changes in enzyme activity from baseline under conditions of chronic stress.

Group	Group Number	SOD (μmol/min g tissue)		GPO (mmol/min g tissue)		AST (mmol/min g tissue)		ALT (mmol/min g tissue)	
		Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver
Control group	1	42.86% (31.79% ÷ 64.55%) *	32.95% (22.44% ÷ 53.55%) *	4.63% (0% ÷ 13.84%) *	40.92% (29.92% ÷ 62.47%) *	623.08% (495.42% ÷ 873.28%) *	267.83% (220.42% ÷ 360.74%) *	479.07% (383.78% ÷ 665.84%) *	183.41% (155.75% ÷ 237.62%) *
0.9% NaCl solution	2	32.53% (22.05% ÷ 53.06%) *	50.11% (38.93% ÷ 72.03%) *	10.26% (3.47% ÷ 23.55%) *	29.84% (19.61% ÷ 49.90%) *	463.81% (371.96% ÷ 643.84%) *	192.31% (162.52% ÷ 250.70%) *	693.37% (549.94% ÷ 974.49%) *	239.36% (198.52% ÷ 319.41%) *
Inhibitor of the enzyme gamma-butyrobetaine hydroxylase	3	116.00% (106.37% ÷ 134.88%) 1,2	114.33% (105.28% ÷ 132.07%) 1,2	96.40% (92.23% ÷ 104.56%) 1,2	112.90% (104.37% ÷ 129.63%) 1,2	119.73% (108.86% ÷ 141.03%) *,1,2	101.74% (98.76% ÷ 107.57%) 1,2	97.64% (94.24% ÷ 104.29%) 1,2	110.19% (102.70% ÷ 124.88%) 1,2
Ligusticum wallichii extract	4	115.56% (106.08% ÷ 134.14%) 1,2	56.00% (44.90% ÷ 77.76%) *,3	115.25% (105.87% ÷ 133.62%) 1.2	216.81% (181.23% ÷ 286.56%) *,1–3	270.00% (222.09% ÷ 363.90%) *,1–3	158.13% (136.69% ÷ 200.15%) *,1.3	51.70% (40.53% ÷ 73.60%) *,1–3	547.96% (437.17% ÷ 765.10%) *,1–3
Eleutherococcus extract	5	126.09% (113.26% ÷ 151.22%) *,1,2	97.30% (93.67% ÷ 104.40%) 1,2,4	94.77% (89.79% ÷ 104.53%) 1,2	104.88% (99.82% ÷ 114.79%) 1,2,4	97.82% (94.56% ÷ 104.22%) 1–4	91.09% (84.72% ÷ 103.58%) 1,2,4	107.96% (101.40% ÷ 120.80%) 1,2,4	100.16% (99.26% ÷ 101.94%) 1,2,4

Notes. Here and below $p < 0.05$; *—differences from the initial level of enzyme activity;1–4—differences from the level of enzyme activity in animals of the corresponding group.

4. Discussion

An increase in AST activities in relation to ALT, which was observed in the rats of the control group under acute stress conditions, may be a sign of damage to myocardial cells. In a number of studies, these enzymes have been used to study the adaptive capabilities of rats in swimming conditions [31–33], as well as with other intense physical activities [34]. The use of these enzymes is due to the fact that intense physical activity can stimulate cytolysis [32].

At the same time, against the background of the enzyme gamma-butyrobetaine hydroxylase inhibitor use, such changes did not occur, which makes it possible to interpret its action as cardioprotective. The effect of Ligusticum wallichii extract under acute stress conditions is, in many respects, similar to the enzyme gamma-butyrobetaine hydroxylase inhibitor, that is, this phytopreparation can also be considered as a cardioprotector under acute stress conditions. The mechanism of such influence remains open. So, it was found that the extracts of Ligusticum wallichii can protect the brain from ischemia, due to docking [35]. It cannot be excluded that the cardioprotective properties of the extracts have the same mechanism. However, this phenomenon needs further study.

Under subacute stress in the rats of the control group, a decrease in the activity of antioxidant enzymes may indicate a violation of oxidative stress processes. An increase in the activities of ALT and AST, in comparison with the initial level, may be a sign of cells destruction both of liver and myocardium. At the same time, due to the increase in the AST/ALT ratio, an assumption can be made about the predominant destruction of myocardial cells. The enzyme gamma-butyrobetaine hydroxylase inhibitor neutralizes these negative changes. At the same time, the effects of two plant extracts explored in the work practically did not differ from the enzyme gamma-butyrobetaine hydroxylase inhibitor. Thus, it can be assumed that, under subacute stress conditions, the enzyme gamma-butyrobetaine hydroxylase inhibitor, as well as the extracts of Ligusticum wallichii and Eleutherococcus, have a cytoprotective effect. It should be noted that for extracts of Ligusticum wallichii under stress, antioxidant effects are described [36]. The mechanism of the influence of Eleutherococcus may be associated with the stimulation of muscle workability [37].

In chronic stress conditions, one can speak of a violation of the processes of antioxidant cells protection against the background of increasing processes of cytolysis. In this case, the inhibitor of the enzyme gamma-butyrobetaine hydroxylase, as in the case of acute and subacute stress, has a protective effect. Under chronic stress conditions, the effects of Eleutherococcus extract are more similar to those of the enzyme gamma-butyrobetaine hydroxylase inhibitor than the Ligusticum wallichii extract. Apparently, Ligusticum wallichii extract is more cytoprotective against hepatocytes than cardiomyocytes, as evidenced by an increase in the AST/ALT ratio of the heart against the background of the decrease in this ratio for the liver. The mechanism of such influence, in our opinion, does not differ from that in conditions of acute stress. For example, the adaptive effects of a number of plant extracts on isolated brain cells have been shown to be related to the regulation of RNA activity. Thus, at least 88 of the 3516 genes that are regulated by adaptogens were closely associated with adaptive stress response and adaptive stress response signaling pathways (ASRSP), including neuronal signaling associated with corticotropin-releasing hormone, mediated by cAMP, protein kinase A. The extracts have influenced many genes that play a key role in modulating adaptive homeostasis, indicating their ability to alter gene expression to prevent disorders that are caused by stress and aging [38].

It should be noted that the decrease in the activity of the antioxidant enzymes SOD and GPO, under cold stress for eight weeks (chronic cold stress), was previously described for rat erythrocytes [39]. A similar effect during the year, activated SOD in the tissues of the myocardium and inhibited GPO [40]. Apparently, not only the duration of cold exposure is important, but also the analyzed tissue in which the activity of the antioxidant enzymes occurs. For example, swimming for eight weeks led to the activation of SOD in the brain [41]. In our study, we analyzed SOD and GPO in the liver and the heart, allowing

us to assume a relationship between a decrease in the activity of antioxidant enzymes and the activation of cytolysis processes, which are indirectly evidenced by enzymes such as ALT and AST, as well as their ratio. It should be noted that the set of enzymes studied by us in this work is used in a number of studies, to determine the degree of adaptation of rats to physical exertion [32–34]. The ratio of enzyme activity also shows the endurance of animals and their fitness [42].

It should be noted the enzyme gamma-butyrobetaine hydroxylase inhibitor is often used to enhance physical performance. An increase in sports performances has been shown in athletes [28] and test animals [43,44] who are under the influence of this drug; however, its use for athletes has been currently prohibited by WADA [45]. One of the arguments for the ban was the long-term persistence of the active substance in human tissues [46]. It should be noted that the drug was developed in the former USSR and was used for a long time to stimulate physical performance. The correctness of the organization of clinical trials of the drug is discussed [47]. Despite the ban on the use of the drug in people in sports, the study of its neuroprotective effects continues [48].

In testing rats, the enzyme gamma-butyrobetaine hydroxylase inhibitor has a cytoprotective effect against toxic effects [49]. The hepatoprotective and cardioprotective properties of the compound have been shown [50]. The cardioprotective effects of drugs have been described in modeling experimental myocardial ischemia [51]. In experimental ischemia of the central nervous system, the enzyme gamma-butyrobetaine hydroxylase inhibitor has antioxidative and neuroprotective effects [46,52].

The mechanisms of drug action are not fully explored, and its pharmacokinetics are highly variable [53], which complicates the practical use of the enzyme gamma-butyrobetaine hydroxylase inhibitor. Therefore, there is a constant search for other adaptogens, including those on a plant basis. For example, in rats swimming, an increase in the activity of antioxidant enzymes is shown under the influence of Chrysanthellum americanum extract [54].

Earlier, it was found that Eleutherococcus had an anti-ischemic effect on test animals [55]. Under chronic stress exposure of rats, the extracts of this plant normalized the homeostasis parameters [56]. It was shown that a complex phytopreparation, containing Eleutherococcus, had a cardioprotective effect [20]. Unfortunately, a large number of publications on Eleutherococcus are written in Russian, and are not available to the general scientific community. Therefore, a separate review was published on the analysis of the Russian-language literature on this phytopreparation [25], which may contribute to the wider use of Eleutherococcus in experimental and clinical studies.

Ligusticum wallichii is traditionally used in Chinese medicine. A systematic review of animal research revealed antioxidant, cytoprotective, and other properties of extracts of this plant [14]. The effect of Ligusticum wallichii preparations on the expression of Toll-like receptors in rat hepatocytes has been shown [57]. When modeling myocardial ischemia, the extracts of this plant have an antioxidant effect [58]. However, for one of the active compounds isolated from the plant, tetramethylpyrazine, long-term side effects on the myocardium have been described. First of all, arterial hypertension and tachycardia develop, and myocardial blood supply deteriorates.

In conclusion, we note that pilot studies began to appear in the literature, on the use of phytopreparations of athletes to improve adaptive abilities and improve physical performance [59]. Research on the adaptive effects of plant extracts continues [26]. In many studies, the authors pay special attention to the study of the molecular mechanisms of adaptation development [60]. It is likely that further research in this direction will increase the evidence base for the effectiveness of herbal medicines.

5. Conclusions

In general, the data obtained suggest the presence of cytoprotective effects in two phytopreparations that are similar to the enzyme gamma-butyrobetaine hydroxylase inhibitor. At the same time, the effect of the Ligusticum wallichii extract is more pronounced

under acute stress conditions, and the effect of the Eleutherococcus extract, under chronic stress conditions. With subacute stress, the effects of both the phytopreparations are similar. The data obtained in this work provide a basis for studying the effectiveness of using phytopreparations as adaptogens under stress conditions.

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References

1. Liu, J.; Yeo, H.C.; Övervik-Douki, E.; Hagen, T.; Doniger, S.J.; Chu, D.W.; Brooks, G.A.; Ames, B.N. Chronically and acutely exercised rats: Biomarkers of oxidative stress and endogenous antioxidants. *J. Appl. Physiol.* **2000**, *89*, 21–28. [CrossRef] [PubMed]
2. Bierens, J.J.L.M.; Lunetta, P.; Tipton, M.; Warner, D.S. Physiology Of Drowning: A Review. *Physiology* **2016**, *31*, 147–166. [CrossRef] [PubMed]
3. Baker, M.A.; Horvath, S.M. Influence Of Water Temperature On Oxygen Uptake By Swimming Rats. *J. Appl. Physiol.* **1964**, *19*, 1215–1218. [CrossRef]
4. Kaushik, S.; Kaur, J. Chronic cold exposure affects the antioxidant defense system in various rat tissues. *Clin. Chim. Acta* **2003**, *333*, 69–77. [CrossRef]
5. Spasić, M.B.; Saičić, S.; Buzadžić, B.; Korać, B.; Blagojević, D.; Petrović, V.M. Effect of long-term exposure to cold on the antioxidant defense system in the rat. *Free Radic. Biol. Med.* **1993**, *15*, 291–299. [CrossRef]
6. Yuksel, S.; Dilek, A.; Ozfer, Y. Antioxidative and metabolic responses to extended cold exposure in rats. *Acta Biol. Hung.* **2008**, *59*, 57–66. [CrossRef] [PubMed]
7. Bandyopadhyay, U.; Das, D.; Banerjee, R. Reactive oxygen species: Oxidative damage and pathogenesis. *Curr. Sci.* **1999**, *77*, 658–666.
8. Wei, H.; Zhang, R.; Su, Y.; Bi, Y.; Li, X.; Zhang, X.; Li, J.; Bao, J. Effects of Acute Cold Stress After Long-Term Cold Stimulation on Antioxidant Status, Heat Shock Proteins, Inflammation and Immune Cytokines in Broiler Heart. *Front. Physiol.* **2018**, *9*. [CrossRef]
9. Su, Y.; Zhang, X.; Xin, H.; Li, S.; Li, J.; Zhang, R.; Li, X.; Li, J.; Bao, J. Effects of prior cold stimulation on inflammatory and immune regulation in ileum of cold-stressed broilers. *Poult. Sci.* **2018**, *97*, 4228–4237. [CrossRef] [PubMed]
10. Davies, K.; Quintanilha, A.; Brooks, G.; Packer, L. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1198–1205. [CrossRef]
11. Lubkowska, A.; Dołęgowska, B.; Szyguła, Z.; Bryczkowska, I.; Stańczyk-Dunaj, M.; Sałata, D.; Budkowska, M. Winter-swimming as a building-up body resistance factor inducing adaptive changes in the oxidant/antioxidant status. *Scand. J. Clin. Lab. Investig.* **2013**, *73*, 315–325. [CrossRef] [PubMed]
12. Lubkowska, A.; Dołęgowska, B.; Szyguła, Z. Whole-Body Cryostimulation-Potential Beneficial Treatment for Improving Antioxidant Capacity in Healthy Men-Significance of the Number of Sessions. *PLoS ONE* **2012**, *7*, e46352. [CrossRef]
13. Lubkowska, A.; Bryczkowska, I.; Gutowska, I.; Rotter, I.; Marczuk, N.; Baranowska-Bosiacka, I.; Banfi, G. The Effects of Swimming Training in Cold Water on Antioxidant Enzyme Activity and Lipid Peroxidation in Erythrocytes of Male and Female Aged Rats. *Int. J. Environ. Res. Public Health* **2019**, *16*, 647. [CrossRef]
14. Wei, Z.; Dong, C.; Guan, L.; Wang, Y.; Huang, J.; Wen, X. A metabolic exploration of the protective effect of Ligusticum wallichii on IL-1β-injured mouse chondrocytes. *Chin. Med.* **2020**, *15*, 12. [CrossRef] [PubMed]
15. Shao, Z.; Wu, P.; Wang, X.; Jin, M.; Liu, S.; Ma, X.; Shi, H. Tetramethylpyrazine Protects Against Early Brain Injury and Inhibits the PERK/Akt Pathway in a Rat Model of Subarachnoid Hemorrhage. *Neurochem. Res.* **2018**, *43*, 1650–1659. [CrossRef]
16. Sumiyoshi, M.; Kimura, Y. Effects of Eleutherococcus senticosus cortex on recovery from the forced swimming test and fatty acid β-oxidation in the liver and skeletal muscle of mice. *Nat. Prod. J.* **2016**, *6*, 49–55. [CrossRef] [PubMed]
17. Bleakney, T.L. Deconstructing an Adaptogen. *Holist. Nurs. Pract.* **2008**, *22*, 220–224. [CrossRef] [PubMed]

18. Adkar, P.P.; Jadhav, P.P.; Ambavade, S.D.; Bhaskar, V.H.; Shelke, T. Adaptogenic Activity of Lyophilized Hydroethanol Extract of Pandanus odoratissimus in Swiss Albino Mice. *Int. Sch. Res. Not.* **2014**, *2014*, 1–10. [CrossRef]
19. Bocharov, E.V.; Ivanova-Smolenskaya, I.A.; Poleshchuk, V.V.; Kucheryanu, V.G.; Il'enko, V.A.; Bocharova, O.A. Therapeutic Efficacy of the Neuroprotective Plant Adaptogen in Neurodegenerative Disease (Parkinson's Disease as an Example). *Bull. Exp. Biol. Med.* **2010**, *149*, 682–684. [CrossRef]
20. Zhuang, Z.; Wang, Z.-H.; Huang, Y.-Y.; Zheng, Q.; Pan, X.-D. Protective effect and possible mechanisms of ligustrazine isolated from Ligusticum wallichii on nephropathy in rats with diabetes: A preclinical systematic review and meta-analysis. *J. Ethnopharmacol.* **2020**, *252*, 112568. [CrossRef] [PubMed]
21. Song, X.; Dai, J.; Li, H.; Li, Y.; Hao, W.; Zhang, Y.; Zhang, Y.; Su, L.; Wei, H. Anti-aging effects exerted by Tetramethylpyrazine enhances self-renewal and neuronal differentiation of rat bMSCs by suppressing NF-kB signaling. *Biosci. Rep.* **2019**, *39*. [CrossRef] [PubMed]
22. Li, X.; Wang, Q.; Ren, Y.; Wang, X.; Cheng, H.; Yang, H.; Wang, B. Tetramethylpyrazine protects retinal ganglion cells against H2O2-induced damage via the microRNA-182/mitochondrial pathway. *Int. J. Mol. Med.* **2019**, *44*, 503–512. [CrossRef]
23. Lv, P.; Tong, X.; Peng, Q.; Liu, Y.; Jin, H.; Liu, R.; Sun, W.; Pan, B.; Zheng, L.; Huang, Y. Treatment with the herbal medicine, naoxintong improves the protective effect of high-density lipoproteins on endothelial function in patients with type 2 diabetes. *Mol. Med. Rep.* **2016**, *13*, 2007–2016. [CrossRef]
24. Lewis, W.H.; Zenger, V.E.; Lynch, R.G. No adaptogen response of mice to ginseng and eleutherococcus infusions. *J. Ethnopharmacol.* **1983**, *8*, 209–214. [CrossRef]
25. Gerontakos, S.; Taylor, A.; Avdeeva, A.Y.; Shikova, V.A.; Pozharitskaya, O.N.; Casteleijn, D.; Wardle, J.; Shikov, A.N. Findings of Russian literature on the clinical application of Eleutherococcus senticosus (Rupr. & Maxim.): A narrative review. *J. Ethnopharmacol.* **2021**, *278*, 114274. [CrossRef] [PubMed]
26. Panossian, A.; Seo, E.-J.; Efferth, T. Effects of anti-inflammatory and adaptogenic herbal extracts on gene expression of eicosanoids signaling pathways in isolated brain cells. *Phytomedicine* **2019**, *60*, 152881. [CrossRef]
27. Kim, Y.-J.; Mathiyalagan, R.; Markus, J.; Wang, C.; Singh, P.; Ahn, S.; Farh, M.E.-A.; Yang, D.C.; Abbai, R. Green synthesis of multifunctional silver and gold nanoparticles from the oriental herbal adaptogen: Siberian ginseng. *Int. J. Nanomedicine* **2016**, *11*, 3131–3143. [CrossRef]
28. Smirnova, M.D.; Svirida, O.N.; Vitsenia, M.V.; Kuzmina, A.E.; Lankin, V.Z.; Tikhaze, A.K.; Konovalova, G.N.; Ageev, F.T. Using meldonium to improve the adaptation of patients with cardiovascular disease to the effects of heat and correction of associated oxidative stress. *Kardiologiia* **2014**, *54*, 53–59. [CrossRef]
29. Baulin, S.I.; Rogacheva, S.M.; Afanaseva, S.V.; Zabanova, E.V.; Karagaycheva, Y. V Pharmaceutical Composition for Improving Physical Working Capacity. *Bull. Exp. Biol. Med.* **2015**, *160*, 45–48. [CrossRef]
30. Baulin, S.I.; Rogacheva, S.M.; Afanaseva, S.V. Effects of Drugs on Exercise Performance. *Bull. Exp. Biol. Med.* **2013**, *155*, 636–638. [CrossRef]
31. Zhang, W.; Su, W.; Lin, Q.; He, Y.; Yan, Z.; Wang, Y.; Li, P.; Wu, H.; Liu, H.; Yao, H. Protective effects of Naoxintong capsule alone and in combination with ticagrelor and atorvastatin in rats with Qi deficiency and blood stasis syndrome. *Pharm. Biol.* **2020**, *58*, 1006–1022. [CrossRef]
32. Farzanegi, P.; Abbaszadeh, H.; Farokhi, F.; Rahmati-Ahmadabad, S.; Hosseini, S.A.; Abdi, A.; Mazandarani, M.R.; Rezaei, I.; Shokrie, M.; Vizvari, E.; et al. Attenuated Renal and Hepatic Cells Apoptosis Following Swimming Exercise Supplemented with Garlic Extract in Old Rats. *Clin. Interv. Aging* **2020**, *15*, 1409–1418. [CrossRef]
33. Chen, Y.-M.; Huang, C.-C.; Hsiao, C.-Y.; Hu, S.; Wang, I.-L.; Sung, H.-C. Ludwigia octovalvis (Jacq.) raven extract supplementation enhances muscle glycogen content and endurance exercise performance in mice. *J. Vet. Med. Sci.* **2019**, *81*, 667–674. [CrossRef]
34. Suarsana, I.N.; Utama, I.H.; Kardena, I.M. Tempe extract reduces cell damage in the liver and kidneys after intensive physical exercise in rats. *Vet. World* **2020**, *13*, 1510–1516. [CrossRef]
35. Song, X.; Zhou, W.; Chen, C.; Wang, S.; Liang, S. [Study on material base of Ligusticum wallichii for treating brain ischemia and its molecular mechanism based on molecular docking]. *China J. Chin. Mater. Med.* **2015**, *40*, 2195–2198.
36. Wang, J.; Liu, N.; Zhang, F. Tetramethylpyrazine Protects Oxidative Stability and Gelation Property of Rabbit Myofibrillar Proteins. *Food Sci. Anim. Resour.* **2019**, *39*, 623–631. [CrossRef] [PubMed]
37. Miyauchi-Wakuda, S.; Kagota, S.; Maruyama-Fumoto, K.; Shiokawa, Y.; Yamada, S.; Shinozuka, K. Acanthopanax senticosus Root Extract Exerts Dual Action on Mouse Ileal Smooth Muscle Function, Leading to Modulation of Gastrointestinal Motility. *Biol. Pharm. Bull.* **2020**, *43*, 817–822. [CrossRef]
38. Panossian, A.; Seo, E.-J.; Efferth, T. Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using systems biology. *Phytomedicine* **2018**, *50*, 257–284. [CrossRef] [PubMed]
39. Gündüz, F.; Sentürk, U.; Kuru, O.; Aktekin, B.; Aktekin, M. The Effect of One Year's Swimming Exercise on Oxidant Stress and Antioxidant Capacity in Aged Rats. *Physiol. Res.* **2004**, *53*, 171–176.
40. Nonato, L.F.; Rocha-Vieira, E.; Tossige-Gomes, R.; Soares, A.A.; Soares, B.A.; Freitas, D.A.; Oliveira, M.X.; Mendonça, V.A.; Lacerda, A.C.; Massensini, A.R.; et al. Swimming training attenuates oxidative damage and increases enzymatic but not non-enzymatic antioxidant defenses in the rat brain. *Braz. J. Med. Biol. Res.* **2016**, *49*, e5310. [CrossRef] [PubMed]
41. Greenblatt, H.K.; Greenblatt, D.J. Meldonium (Mildronate): A Performance-Enhancing Drug? *Clin. Pharmacol. Drug Dev.* **2016**, *5*, 167–169. [CrossRef]

42. Chang, Q.; Miao, X.; Ju, X.; Zhu, L.; Huang, C.; Huang, T.; Zuo, X.; Gao, C. Effects of Pulse Current on Endurance Exercise and Its Anti-Fatigue Properties in the Hepatic Tissue of Trained Rats. *PLoS ONE* **2013**, *8*, e75093. [CrossRef] [PubMed]
43. Seĭfulla, R.D.; Trevisani, K.; Morozov, I.E.; Kondrat'eva, I.I.; Fedina, T.I.; Trevisani, M.; Agaeva, E.N. The action of carnitine derivatives on the work capacity of previously trained animals. *Eksp. Klin. Farmakol.* **1993**, *56*, 34–36.
44. Hughes, D. Comment: Meldonium and the WADA Prohibited List. *Aust. Prescr.* **2016**, *39*, 102. [CrossRef] [PubMed]
45. Lykhatskyi, P.; Fira, L.; Garlitska, N.; Fira, D.; Soroka, Y.; Lisnychuk, N.; Delibashvili, D. CHANGES OF CYTOLYSIS Indicators in rats' blood resulted from simultaneous intoxication with tobacco smoke and sodium nitrite after using mildronate. *Georgian Med. News* **2019**, *296*, 96–102.
46. Rabin, O.; Uiba, V.; Miroshnikova, Y.; Zabelin, M.; Samoylov, A.; Karkischenko, V.; Semyonov, S.; Astrelina, T.; Razinkin, S. Meldonium long-term excretion period and pharmacokinetics in blood and urine of healthy athlete volunteers. *Drug Test. Anal.* **2019**, *11*, 554–566. [CrossRef] [PubMed]
47. Chirkova, A.; Petrenko, A.; Vasilyev, P. Testing Meldonium: Assessing Soviet pragmatic alternatives to the randomized controlled trial. *Clin. Trials* **2021**, *18*, 269–276. [CrossRef]
48. Tanashyan, M.M.; Maksimova, M.Y.; Shabalina, A.A.; Fedin, P.A.; Medvedev, R.B.; Bolotova, T.A. Chronic cerebrovascular diseases and neuroprotection: The clinical efficacy of meldonium (Mildronat). *Zhurnal Nevrol. i Psikhiatrii im. S.S. Korsakova* **2020**, *120*, 14. [CrossRef]
49. Liepinsh, E.; Kuka, J.; Svalbe, B.; Vilskersts, R.; Skapare, E.; Cirule, H.; Pugovics, O.; Kalvinsh, I.; Dambrova, M. Effects of Long-Term Mildronate Treatment on Cardiac and Liver Functions in Rats. *Basic Clin. Pharmacol. Toxicol.* **2009**, *105*, 387–394. [CrossRef]
50. Kukes, V.G.; Zhernakova, N.I.; Gorbach, T.V.; Romashchenko, O.V.; Rumbesht, V.V. Efficiency Of Mildronate In Rats Of Different Age With Experimental-Induced Myocardial Ischemia. *Ann. Russ. Acad. Med. Sci.* **2013**, *68*, 42–46. [CrossRef]
51. Demir, D.; Kuru Bektaşoğlu, P.; Koyuncuoğlu, T.; Kandemir, C.; Akakın, D.; Yüksel, M.; Çelikoğlu, E.; Yeğen, B.Ç.; Gürer, B. Neuroprotective effects of mildronate in a rat model of traumatic brain injury. *Injury* **2019**, *50*, 1586–1592. [CrossRef]
52. Sokurenko, L.M.; Savchyna, M.O.; Litus, V.I.; Kaminsky, R.F.; Chaikovsky, Y.B. Rat spinal ganglia in assessment of protective action of antioxidants: A morphological study. *Medicina* **2017**, *53*, 316–322. [CrossRef]
53. Cojocariu, R.; Ciobica, A.; Balmus, I.-M.; Guenne, S.; Trifan, A.; Stanciu, C.; Hriţcu, L.; Lefter, R. Antioxidant Capacity and Behavioral Relevance of a Polyphenolic Extract of Chrysanthellum americanum in a Rat Model of Irritable Bowel Syndrome. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 1–13. [CrossRef] [PubMed]
54. Wang, R.; Shi, L.; Liu, S.; Liu, Z.; Song, F.; Sun, Z.; Liu, Z. Mass spectrometry-based urinary metabolomics for the investigation on the mechanism of action of Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. leaves against ischemic stroke in rats. *J. Ethnopharmacol.* **2019**, *241*, 111969. [CrossRef]
55. Shakhmatov, I.I.; Bondarchuk, I.A.; Vdovin, V.M.; Alekseeva, O.V.; Kiselev, V.I. Hemostasis changes and their correction by adaptogen. *Patol. Fiziol. Eksp. Ter.* **2010**, 43–46.
56. Lishmanov, I.B.; Maslov, L.N.; Arbuzov, A.G.; Krylatov, A.V.; Platonov, A.A.; Burkova, V.N.; Kaiumova, E.A. Cardioprotective, inotropic, and anti-arrhythmia properties of a complex adaptogen "Tonizid. *Eksp. Klin. Farmakol.* **2008**, *71*, 15–22. [PubMed]
57. Zengyong, Q.; Jiangwei, M.; Huajin, L. Effect of Ligusticum wallichii Aqueous Extract on Oxidative Injury and Immunity Activity in Myocardial Ischemic Reperfusion Rats. *Int. J. Mol. Sci.* **2011**, *12*, 1991–2006. [CrossRef]
58. Chiou, G.C.; Yan, H.Y.; Lei, X.L.; Li, B.H.; Shen, Z.F. Ocular and cardiovascular pharmacology of tetramethylpyrazine isolated from Ligusticum wallichii Franch. *Zhongguo Yao Li Xue Bao* **1991**, *12*, 99–104. [PubMed]
59. Henkel, R.R.; Wang, R.; Bassett, S.H.; Chen, T.; Liu, N.; Zhu, Y.; Tambi, M.I. Tongkat Ali as a Potential Herbal Supplement for Physically Active Male and Female Seniors-A Pilot Study. *Phyther. Res.* **2014**, *28*, 544–550. [CrossRef]
60. Seo, E.-J.; Klauck, S.M.; Efferth, T.; Panossian, A. Adaptogens in chemobrain (Part III): Antitoxic effects of plant extracts towards cancer chemotherapy-induced toxicity-transcriptome-wide microarray analysis of neuroglia cells. *Phytomedicine* **2019**, *56*, 246–260. [CrossRef]