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Research Article
Adaptable properties of bioactive additive "Grail"

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Abstract

The adaptive characteristics of a bioactive additive "Grail" is researched and it is shown, that in an interval of 1-10 ml/kg doses, it noticeably increases the body's resistance to stress, physical overcharges, poisoning by the hypoxic poison, deficiency of oxygen, fire-fanging and an activity of bacterial endotoxin. In the same interval of doses , it exposes a clear-cut immuno-modulate feature – it stimulates T-dependence proliperation and increases the functional activity of phagocytes. Immuno-modulated component exposes adaptive ascendancy on the non-specific resistance of the organizm. The totality of the given features of the product can be designated by the term "common intensifier".

Introduction

Bioactive additive "Grail" is created under the Transnational program "Chernobyl - help" as means for preventive maintenance and treatment of radiation injuries. It represents a mix of extracts more than 30 medical-food plants, products of beekeeping and winemaking [1].

As the preparation radioprotective actions is registered in Ministries of Health of Georgia, Ukraine and Byelorussia [2-4]. Besides antiradiative properties of product possesses a wide spectrum of other medical action [5-10].

In given article results of research of product on adaptogenic properties are resulted.

The most valuable property of tea, ginseng, eleutherococcus, a gold root, antlers of dapple deer and other components included in "Grail", ability to raise vitality of an organism is at overloads, coolings, overheating and to that the similar influences showing to an organism increased requirements. This property confirmed with the numerous experimental and clinical data, has received the name adaptogenic effect, under which it is meant to raise ability of medicinal substances nonspecific resistency (resistibility, stability) of an organism to influence the physical, chemical and biological factors causing infringement of a homeostasis and leaders to diseases. As a basis of tested product are tea, a gold root, a ginseng and eleutherococcus, enriched with additional vegetative components with cardiotrophical and other kinds of biological activity, the estimation adaptogenic properties of a "Grail" is lead to conformity with an expected structure of its pharmacological efficiency, namely, are investigated adaptogenic properties of a product at extreme (stressful) influences, oxygen starvation, the overheating, the raised loadings on system of blood circulation.

Materials and Methods

Anti-stressful action

It is known, that various pathological conditions and extreme influences cause the common adaptable reaction of an organism shown by standard triad Sale - a hypertrophy of a bark of adrenal glands, involution thimic-lymphatic bodies and detectioning in a gastroenteric path. Pharmacological means and phytopreparations - regulators of stress (adaptogenes) at preliminary introduction raise nonspecific resistibility of an organism to extreme influences and weaken reaction to stress from its humous and morphological consequences.

For an estimation of efficiency of bioactive addition as potential antistressful preparation in experiences on rats influence of repeated introduction of a "Grail"(BAG) on displays of the stress caused long immobilization has been investigate.

Experiments are carried out on 36 male rats Vistar in weight of 115-130 g whom during 7 days up to stressful influences intragastralicaly entered a preparate in dozes of 1 and 3 ml / kg. Control rats in quality placebo received water (0.3 ml / 100 g), and the biological control - only water and stress was not exposed.

Stress caused rigid fixing of rats on a back at 24 o'clock. After expiry of the term of fixing of animals hammered decapitation, took and weighed adrenal glands, thimus, a spleen. In adrenal glands determined the maintenance of an ascorbic acid - one of the most sensitive exponents of activation of synthesis of glucocorticoids (reaction with dichlorphenolindiphenole). Macroscopiocaly estimated a condition mucous a stomach and an intestines, determined the contents of sugar in blood.

Preventive maintenance of physical overfatigue

It is known, that excessive physical loadings at regular recurrence result, as a rule, in an increasing exhaustion of physical strengths, and at times and to irreversible pathological changes in a cardiac musle both other bodies and systems. Adaptogenes, as against neirostimulators mobilized such as action (fenamine, syndocarb and means similar to them), should raise stability of an organism in conditions of such chronic overloads. In this connection for an estimation adaptogenic properties of a BAG the model of regular overloads at rats - daily run on trainbass with the submaximal speed up to full exhaustion has been used.

Influence of a BAG on stability to regular physical overloads is investigated in experiments on rats under the test of high-speed endurance at run on trainbass.

The test carried out on trainbass with automatic maintenance of the set speed of movement of pathes within 0.1 m / minutes. Serviceability estimated on time of run up to exhaustion (at speed of 32 m / mines) as which criterion repeated landing of animals to an electrode floor and other attributes of weariness served. For experiment selected standardized male rats (weight 120-130 g) after their preliminary training to skills of run in trainbass at the lowered speed. The selected animals shared on equivalent control and experimental groups on 10 individuals.

BAG entered to experimental rats in dozes of 1 and 3 ml / kg intragastralicaly 1 hour prior to tests. Control rats received inside water in the same volume. Re-testing on serviceability carried out daily within 10 day. At every research rats received a preparation in the same dozes for 1 hour before run.

Antihypoxical properties

Stability of an organism to hypoxia - one of the most integrated parameters of a functional condition of various physiological systems and biopower processes. For an establishment of effects of BAG on resistency to hypoxia models hypobaric and hemic hypoxia, describing various mechanisms of realization antihypoxical effect are used.

Hemic hypoxia

.Experiences are lead on female mice (CBA x C_{57}BL) F1. Hemic hypoxia caused nitrite of sodium in a fatal doze of 300 mg / kg. A of BAG 10 and 30 ml / kg for 1 entered unitary in dozes 3, hour before introduction of nitrite of sodium or repeatedly in dozes of 3 and 10 ml / kg during 7 days. Antihypoxical effect estimated on a survival time of animals at a fatal poisoning.

Hypobaric hypoxia

Influence of BAG on stability of mice to hypobaric hypoxia was estimated on a survival time of animals in a rarefied atmosphere at "height" of 11 kms.

Influence on stability to overheating

In experiences on 20 Vistar female rats in weight 220-250 g estimated influence of BAG on reactance of cardiovascular system and breath at total convectional overheating.

Animals preliminary adapted for conditions of measurement of arterial pressure and pulse oscillative a method on tail arteries at temperature 36^0C. Thermal stress (41^0C) caused a stream of heated up air in the thermal chamber at the control of temperature of a stream over accuracy 0.2^0C. The BAG was entered 1 hour prior to tests in a doze of 10 ml / kg.

Antiendotoxic action

It is known, that biological effects endotoxine – gramme-negative lipopolisaccharide the bacteria causing heavy infectious defeats (a belly typhus, a dysentery, salmonellosises) and toxico-infections, are caused by clearing an organism from lymphocites and macrophages endogenic citokieve – tumor necrotive factor (TNF) responsible for the majority of displays of toxic action endotoxine in an organism (frustration hemodinamical, hypercoagulation of blood, a shock and destruction of animals). It is known as, that adaptogene of the vegetative and synthetic nature raise resistency to bacterial invation and endotoxine. One of possible mechanisms of this protective effect - blockade of clearing TNF, involutive endotoxine.

For an estimation protective properties of the BAG containing a set effective natural adaptogenes, in experiments on mice influence of a product on involutive endotoxine clearing TNF has been investigated.

Experiments are lead on hybrid mice - males in weight of 18-20 g. The BAG was entered an animal intragastralicaly in

dozes of 1 and 3 ml / kg within 6 day. As the positive control used a powder of roots of a ginseng in a doze of 10 mg / kg and the control on placebo - water . For 7 day all animal intravenously entered endotoxine in a doze 0.5 mg / mouse. In 1 hour of animals hammered decapitation for reception of whey of blood in which determined credit TNF citotoxic the test for monolayer culture mouse fibroblasts . For unit of activity accepted the cultivation of whey (EC_{50}) causing 50 % destruction of cells in culture. Individual and middlegroups values EC_{50} determined regressive analysis with calculation of confidential intervals at P = 0.05.

Immunomodulative activity

Presence in structure of BAG of the vegetative components possessing immunomodulative effects, was the basis for studying immunotrophic actions of a product on influence on production antibodycounter cells (ABC) and fagocitary activity which on the known data are sensitive to various phytopreparation.

Stimulation of B-cells

Influence of a BAG on a condition of antibody estimated in experiments on hybrid mice in weight 21-23 g by amount of antibodycounter cells (ABC) in a spleen at immunization of roulea in the ram (3×10^8 cells). Immunization was carried out in 1 day after the termination of a 6-day's rate of introduction of a BAG in dozes of 3 and 10 ml / kg or placebo (water) intragastralicaly. The amount of ABC in a spleen determined method Canningheim in 4 day after introduction of an antigene.

Influence on phagocytes

Experiments are carried out on culture peritonitist the macrophages received from control and skilled mice after 6 - day time introduction of a BAG in dozes of 1 and 3 ml / kg. Phagocyte activity determined on intensity of restoration of nitroblue tetrozole (NBT) in reply to stimulation phagocyte process zimozan. Peritonal acavity washed off environment 199 with addition of 10 % of whey of large horned livestock, on $5 \times I0^6$ cells, landed on plastic cups Petrie in diameter 3.5 sm and we were incubating 2 hours at 37^0C. After an attachment of cells environment replaced fresh, containing zimozan (50 mg / ml), incubated 30 minutes and then brought NBT (10 mg / ml) at the presence of which incubation proceeded 60 minutes. Dye extragated 1 M NaOH and optical density of an extract measured at 675 nanometers. In control cups zimozan did not bring or incubative tests with zimozan carried out at 4^0C.

Results

Antistressful action. Results of researches are submitted in tab. 1.

It is established, that immobilized stress in the given updating causes in control rats distinctly expressed displays of reaction of a pressure - a hypertrophy of adrenal glands with falling the maintenance in them of an ascorbic acid, involution of thimus and spleens, hyperglicemia and detection of mucous a stomach.

Table 1. Influence of a BAG (3 ml / kg) 1) on metabolic and organ displays of stress at the rats, caused immobilization

Parameters	Biological control	Stress	
		Placebo	BAG
Adrenal glands, mg%	22.7±I.2	$29.3 \pm 1.6^{+}$	25.4 ± 1.3^{U}
Ascorbic acid-that adrenal glands, mg%	852±19	$564 \pm 17^{+}$	$681 \pm 19^{*+}$
Sugar in blood, mg%	115± 5	$157 \pm 6^{+}$	130± 5*
Thimus, mg%	174± 8	$109 \pm 6^{+}$	$137 \pm 7^{*+}$
Frequency of cases Detection of a stomach	0/9	8/9	$4/9^{T}$

Notes: [1)] the BAG entered within 7 days in groups - on 9 rats; $^{+}$ - Authentic variously with biological control ($P \leq 0.05$); * - the same in comparison with placebo by criterion t-Student; ^{u} - the same by U-criterion; ^{T}- the same on TMF.

Introduction of a BAG in a doze of 3 ml / kg essentially weakens the above-stated displays of stress: the hypertrophy of adrenal glands decreases approximately on 2/3 and decrement of an ascorbic acid is accordingly reduced, hyperglicemia almost is completely levelled, falling weight lymphatic bodies is authentically weakened. Frequency of development detection mucous a stomach is reduced from 90 % up to 45 % ($P \leq 05$).

In a doze of 1 ml / kg day BAG essentially has not affected on specified metabolic and organic displays of stress at rats. Thus, BAG at course introduction possesses distinct antistressful activity estimated on hormonal and organic to displays of reaction of a pressure. The essence antistressful effect of this multicomponent vegetative preparation, probably, consists that BAG at repeated introduction

induces a condition of the increased specific resistibility of an organism and in this connection weakens a level alarm - reactions (hypersecretion of glucocorticoids) to injuring stimulus. In the given attitude balm simulates effects known as adaptogenes.

Preventive maintenance of physical overfatigue. Results of researches are submitted in table 2 where average values and medians of time of run of rats up to exhaustion are resulted daily during 10-day's experiment. It is established, that at the first test the concentrate essentially does not influence on average time of run up to exhaustion, however, further advantage of animals, receiving the preparation before work, stably accrues. Actually up to 8 - 9-th day of daily loadings at experimental rats average time of run increases and at the greater half of these animals in 1.5-2 times absolute parameters of serviceability grow. The share of experimental rats at which overfatigue (time of run develops falls below initial), makes 20-30 %.

Table 2. Influence of daily introduction BAG on duration of run up to exhaustion at rats at 10- day's tests (minutes, M±m, n=10)

Days of tests	Placebo	A BAG in dozes, ml / kg	
		1	3
1	13.2±0.9 (13)	13.4±1.0 (13)	13.4± 2.3 (13)
2	18.6±1.6 (18)	22.4±2.5 (20)	20.1± 3.4 (20)
3	25.6±4.5 (21)	27.4±4.0 (28) *	34.2± 7.2 (28) *
4	26.8±7.6 (19)	33.2±8.3 (29) *	36.6± 7.6 (30) *
5	24.5±8.0 (9)	32.2±7.8 (22) *	34.1±10.9 (20) *
6	17.1±71 (3)	34.4±8.0 (25) *	37.7± 8.5 (25) *
7	11.1±5.0 (3)	34.1±7.7 (24) *	24.0± b.8 (! b) *
8	11.9±4.9 (3)	24.4±5.2 (17) *	20.0± 4.1 (13) *
9	3.6±1.4 (3)	17.2±3.1 (13) *	21.4± 9.3 (7) *
10	4.2±2.2 (0)	14.4±3.1 (10) *	13.1 + 5. 3 (4) *

*Notes: values of a median are specified in brackets; * - authentic distinctions with control (P≤05) over criterion Wilcocson-Manna-Witny.*

At control rats other dynamics of serviceability is observed at regular overloads. After 3-5-ro day of tests serviceability at the majority of them falls below initial down to zero; in a result half of rats (see values of medians) after 5-th day maintains loading of no more than three minutes and only at separate animal (30 %) arise some training effect and time adaptation to loading (up to 7-8-th day).

The BAG possesses the maximal stimulating effect in a doze of 3 ml / kg. In this case the high serviceability exceeding an initial level, is kept at the greater half of rats till 8-th day continuous exhaustible loadings. At a doze of 1 ml/kg the stimulating effect on serviceability is a little bit lower, however, adaptogenic action thus longer period, down to 10-th day when the majority of control rats practically became completely incapacitated is shown.

Thus, BAG renders expressed adaptogenic action at regular exhaustible loadings, providing preservation of high serviceability at repeated tests in operating conditions up to limiting exhaustion.

Antihypoxical properties. Results of researches are submitted in tab. 3.

Apparently from tab. 3, BAG renders antihypoxical action on model hemic hypoxia at unitary introduction in dozes of 10 and 30 ml / kg, but is more effective at repeated introduction.

Table 3. Protective effect of BAG on model sharp hemic hypoxia

Groups	Dozes, ml / kg	ALE, minutes [1)]	P	An index of
The control	-	18.5 ±1.1		
Unitary introduction	3	20.3 ±1.5		1.09
	10	23.4±1.8	<0.05	1.26
	30	25.7±2.2	<0.05	1.39
The control	-	17.6+0.8		
7-day's introduction	3	23.7 ±1.4	<0.05	1.34
	10	28.9±1.7	<0.01	1.64

[1)] - ALE - average life expectancy

Hypobarric hypoxia.

It is established (tab. 4), that on the given model BAG at unitary introduction in dozes of 10 and 30 ml / kg increased life expectancy of animals over 20 minutes in 100 % of cases at 14.6±2.4 minutes in the control and raised percent of survival rate in hypobaric conditions first 30 minutes of an exposition with 40 up to 80-100 %. In an interval of the tested dozes the preparation did not change a body temperature of animals.

Table 4. Influence of BAG on stability of mice to sharp hypobaric hypoxia

Dozes of ml / kg	n	ALE, mines	Survival rate, %
The control (water)	10	14.6 ± 2.4	40
3.0	10	18.4±2.3	60
10.0	10	> 20 *	80*
30.0	10	> 20 *	100*

P≤05 in comparison with the control

Presence at BAG protective properties revealed on classical models hypoxia, at absence of essential influence on the basic exchange, testifies, that BAG raises stability of life-support systems of animals in conditions of extreme oxygen deficiency and can be related to moderately effective antihypoxical to means.

Influence on stability to overheating

It is established, that thermal heating causes in the control animals who have received inside water in volume of 10 ml / kg, a sharp hypertensia and tachycardia with the subsequent failure of functional stability of cardiovascular system and development of a thermal shock within the first hour. Introduction of a BAG slowed down approach hemodynamic failures and reduced frequency of cases of a shock by 33 % (at 2 of 6 rats) at overheating within one hour.

Antiendotoxic action

Results of studying antiendotoxic actions of BAG under the test of induction TNF are submitted in tab .5.

Table 5. Inhibit influence of a tested BAG and a substance of roots of a ginseng on induced by endotoxine clearing TNF at mice

№	Groups	Number of mice	Credit TNF(EU - 50)	Inhibition percent[1)]
1.	The biological control	6	<10	
2.	Placebo + endotoxine	10	409 (365-459)	-
3.	A ginseng of 10 mg / kg + endotoxine	6	177 (142-222) *	57
4.	BAG 1 ml / kg + endotoxine	6	217 (189-250) *	47
5.	BAG 3 ml / kg + endotoxine	6	107 (75-154) **	74

- the Percent of on inhibition is designed under the formula $(T_1 – T_2)/T_1$ x 100, where T_1 - credit TNF at isolated introduction endotoxine (group 2), T_2 - a credit on a background of preparations.
** - P ≤0.01; ** - P ≤ 0.001 in comparison with endotoxine (placebo)*

The received data show, that the tested concentrate and a substance of roots of a ginseng at preliminary introduction to mice during 6 days cause the expressed decrease in sensitivity of animals to action endotoxine - suppress clearing endogenic TNF.
It is remarkable, that effects of a ginseng in a doze of 10 mg / kg and a BAG in a doze of 1 ml / kg practically coincide. The extract in a doze of 3 ml / kg possesses the essentially greater activity on a degree of inhibition clearing TNF.

Thus, the ginseng and a BAG in structure of extracts of a ginseng, of eleutherococcus and other components, possess expressed antiendotoxine effect - inhibit caused endotoxine clearing TNF in an organism.

The BAG under this test is more effective, than equivalent dozes of a ginseng that specifies complex action of vegetative substances included in a composition. Antiendotoxine action of a ginseng and a BAG is shown in dozes which enter into an interval of recommended dozes for the person.

The carried out researches allow to conclude, that regular application of BAG can result in increase of stability of an organism to the effects caused by bacterial endotoxines.

Stimulation of B-cells

It is established (tab. 6), that 6-day's introduction of a BAG in both tested dozes renders stimulating influence on process of generation antibodycounter cells which output raises on the average in 1.5 times in comparison with the control. The similar effect of stimulation can be estimated as moderately aduvantive.

Table 6. Influence of 6-day's introduction BAG on quantity antibodycounter cells in a spleen at mice, immunized by roulea in the ram (M±m, n=10)

Doze, ml / kg in day	Weight of a spleen, mg	ABC x $I0^3$
Placebo	126± 6	232± 8
3.0	139±11	326±15*
10.0	158± 8 *	384±22*

P ≤0.05 by t-criterion Student

Influence on phagocytes

The results submitted in tab. 7, testify, that 6-day's introduction of a BAG in dozes of 1 and 3 ml / kg raises ability phagocytive cells to answer reaction of metabolic activation NADF-pentozic of the shunt, induced by the active forms of oxygen causing bactericidal effect at phagocyte.

Table 7. Influence of 6-day's introduction BAG on phagocytive metabolic reaction on peritonal macrophages, induced by zimozan

Variants of statement of the test	Optical density restored NTB
Macrofags of intactive mice	
1. + zimozan, 37^0C	0.169 ± 0.021
2. + zimozan, 40^0C	0.061 ± 0.012
3. - zimozan, 37^0C	0.055 + 0.010
Macrofags of the experimental mice which have received BG in the specified dozes, stimulized by zimozan at 37^0C With	
4. 1.0 ml / kg	0.267 ± 0.027*
5. 3.0 ml / kg	0.332 ± 0.036*
6. Placebo	0.138 ± 0.021

P ≤0.05 in compared with placebo

Results of the lead researches allow to conclude, that BAG in dozes of an interval renders of 1-10 ml / kg moderately expressed stimulative action on humous and nonspecific immunity.

Discussion

Results of studying adaptive properties of bioactive additive "Grail"(BAG) convincingly testify, that the given composition of medicinal grasses in an interval of dozes of 1-10 ml / kg has property to raise resistence of an organism to stress, physical loadings, a poisoning by hypoxic poisons, to oxygen starvation, overheating and action bacterial endotoxine.

Most remarkable of these effects - adaptogenic action at long immobilize stress which causes strongly pronounced triad Salle in the majority of control animals - a hypertrophy of a bark of adrenal glands and decrease in the contents in them of an ascorbic acid (an attribute of the increased secretion of glucocorticoids), involution thimic-lymphatic system and detect mucous a stomach. The BAG in such conditions provides essential easing metabolic and organ displays of stress that testifies to increase of nonspecific resistency of an organism and decrease under influence of BAG alarm - reactions to injuring influences.

Other remarkable effect of BAG - adaptogenic action at daily physical overloads which at the majority of control animals rather quickly result in overfatigue and full physical incapacity. BAG in such conditions provides not only preservation, but also a gain of serviceability at daily work up to full exhaustion, extending thus the period of an efficient condition of animals.

As all models of overloads used here, intoxications and hypoxic conditions characterize endurance of an organism as a whole and its major life-support systems - biopower processes, functional stability of a brain and is intimate - the vascular system, the received results give the basis to conclude, that BAG adaptogenic means, capable to raise the general nonspecific resistency of an organism to

influence of the various factors causing frustration of a homeostasis.

In an interval of dozes of 1-10 ml / kg the BAG shows moderately expressed immunomodulative properties - stimulates T-dependent proliferation B-cells and raises functional activity of phagocytes. Immunomodulative component can be valuable addition in adaptogenic influence of BAG on nonspecific resistency of an organism.

Set of the described properties of a BAG can be designated by the term of general lining action.

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