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Preliminary experimental research of phytotea "ATINE"

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Summary

Background and aim. Earlier we shown that phytotea "ATINE" enhances the immunomodulatory effect of adaptogenic factors of the Truskavets' Spa in patients after radical treatment of oncological pathology as well as has physiologically beneficial effects on the neuro-endocrine-immune complex and metabolism of patients with maladaptation. The purpose of this study is to find out fundamental effects of "ATINE" in controlled experiments on rats.

Material and methods. The experiments were conducted on 42 outbred white female rats weighing 170 - 250 g. In the first experiment, the object of the study was the weight of the body and individual organs, the activity of AlT and AsT as markers of cytolysis, the levels of erythrocytes, hemoglobin and leukocytes in the blood as well as leukocytogram at intact rats ant treated by daily water or "ATINE" infusion. In the second experiment with a similar design, an open field test and immune test was performed. In the third experiment, the subjects of the study were plasma levels of lipid peroxidation products and the weight of the adrenal glands, thymus, and spleen in intact rats and rats exposed to acute heat stress while consuming daily water or "ATINE".

Results. We first of all stated the absence of general toxic effects of ATINE during 28 days of use. Judging by the *significant* Cohen's effect sizes d_s of the *registered* variables, the antioxidant effect of "ATINE" is 3.37±0.13; stress-limiting 2.97±0.75; erythropoietic 2.67±0.43; neurostimulating 1.95±0.32 and immunostimulating 1.78±0.30.

Conclusion. Phytotea "ATINE" exhibits significantly pronounced classic adaptogen properties.

Keywords: Phytotea "ATINE", female rats, general toxicity, antioxidant, stress-limiting, erythropoietic, neurostimulating and immunostimulating effects.

Introduction

Herbal tea “ATINE” produced by PrJSC "Liktravy" (Zhytomyr, Ukraine). Developer: Bombushkar I.S., MD. Technical conditions 15.8-2811804034-001.2009. International registration No. 1812911 (ATINE). The method of preparing the tea is protected by a patent of Ukraine: (19)UA (11)159836 (13)U (51)МПК *A61K 36118(2006.01)*. 09.07.2025, Bull N28.

Here are the components of the “ATINE”: *Rhizomata Bergeniae, Radices Berberidis, Radix Ononidis, Rhizomata Filipendulae, Rhizomata Bistortae, Radices Geumeris, Rhizomata et radices Inulae, Rhizomata et radices Angelicae, Radices Symphytii, Radices Limonidis, Radices Taraxaci, Rhizomata calami, Radices Bardanae, Fructus Myristici, Fructus Brioniae, Rhizomata tormentillae, Rhizomata Graminis, Radices Iridis pseudacori, Rhizomata et radices Paeoniae anomalae, Radices Althaeae, Rhizomata et radices Rhodiolae quadrifidae, Radices Sanguisorbae, Radices Glycyrrhizae, Radices Cichorii, Radices Rumicis, Hedysarum neglectum.*

According to the data of the SS Institution for single crystals of NAS of Ukraine (Kharkiv), the GC–MS profile (TIC ATINE_MeOH) contains the following substances:

1. Fatty acids and their esters (≈35–40 % of the total area): Myristic acid (C14:0), palmitic (C16:0) and linolenic (C18:3); Isopropyl esters (isopropyl myristate, isopropyl palmitate).

2. Polar phenolic compounds and phenylpropanoids (≈15–20 %): Furfural and its hydroxymethyl derivatives Asarone, 2-methoxy-4-vinylphenol, Osthole (coumarin lactone).

3. Pyrone structures and lactones (≈13–16 %): 4H-Pyran-4-one derivatives; Isoalantolactone.

4. Low molecular weight acids and aldehydes (≈5–10 %): Acetic and benzoic acids.

Total functional load of the sample:

Cellular protection and barrier function — due to saturated fatty acids and their esters. Anti-inflammatory and cardioprotective effect — linolenic acid (ω-3). Antioxidant activity — polyphenols (furfurols, pyrones). Microbiological safety and regulation of microflora — phenolic acids. Antispasmodic and anxiolytic action — Osthole and 4H-pyrones. Potential cytotoxic effect — Isoalantolactone at pharmacological concentrations.

Thus, the chemical composition of the extract combines lipophilic components that strengthen the barrier and energy functions of the body, with polar metabolites that have antioxidant, antimicrobial and anti-inflammatory activity.

Earlier we shown that phytotea “ATINE” enhances the immunomodulatory effect of adaptogenic factors of the Truskavets’ Spa in patients after radical treatment of oncological pathology [4] as well as has physiologically beneficial effects on the neuro-endocrine-immune complex and metabolism of patients with maladaptation [5].

The purpose of this study is to find out fundamental effects of “ATINE” in controlled experiments on rats, to determine the fundamental effects of the herbal tea "ATINE" in

controlled experiments on rats, with particular emphasis on its impact on hematological, immunological, neurobehavioral parameters, and resistance to oxidative and heat stress.

Research Problems

1. Does the administration of "ATINE" infusion affect blood morphology parameters (erythrocytes, hemoglobin, leukocytes) in rats?
2. Does the administration of "ATINE" influence the immune response in the tested animals?
3. Does supplementation with "ATINE" modify the behavior of rats in the open field test (neurobehavioral parameters)?
4. Does "ATINE" exhibit protective effects against oxidative and heat stress in rats?

Research Hypotheses

1. Administration of "ATINE" infusion increases the number of erythrocytes and the level of hemoglobin in rats.
2. Supplementation with "ATINE" enhances the functional activity of the immune system in rats.
3. Administration of "ATINE" improves neurobehavioral parameters (e.g., locomotor activity) in the open field test.
4. "ATINE" limits the negative effects of oxidative and heat stress in rats.

Statistical Hypotheses

For each research problem:

1. Null hypothesis (H0): Administration of "ATINE" does not cause significant changes in the number of erythrocytes and the level of hemoglobin in rats compared to the control group.

   Alternative hypothesis (H1): Administration of "ATINE" causes a significant increase in the number of erythrocytes and the level of hemoglobin in rats compared to the control group.
2. H0: Supplementation with "ATINE" does not significantly affect the functional activity indicators of the immune system in rats.

   H1: Supplementation with "ATINE" significantly increases the functional activity indicators of the immune system in rats.
3. H0: Administration of "ATINE" does not cause significant changes in the neurobehavioral parameters of rats in the open field test.

   H1: Administration of "ATINE" causes a significant improvement in the neurobehavioral parameters of rats in the open field test.
4. H0: "ATINE" does not exhibit a significant protective effect against oxidative and heat stress in rats.

   H1: "ATINE" exhibits a significant protective effect against oxidative and heat stress in rats.

Material and methods

Participants

The experiments were conducted on 42 outbred white female rats weighing 170 - 250 g.

Ethics approval

All animals were kept in room having temperature 22±2ºC, and relative humidity of 44-55% under 12/12 hours light and dark cycle with standard laboratory diet and water given ad libitum. Studies have been conducted in accordance with the rules and requirements of the “General Principles for the Work on Animals” approved by the I National Congress on Bioethics (Kyïv, Ukraine, 2001) and agreed with the provisions of the “European Convention for the Protection of Vertebrate Animals used

for Experimental and other Scientific Purposes" (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine "On the Protection of Animals from Cruelty" of 26.02.2006. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Study design and procedure

Three experiments were conducted.

In the first experiment, 6 animals remained intact, 6 formed a control group, whose members were administered intragastrically daily water (2.5 mL/200g) once for 28 days, and 6 rats of the main group - a similar volume of ATINE infusion. This is twice the recommended dose [37]. The object of the study was the weight of the body and individual organs, the activity of AlT and AsT as markers of cytolysis, the levels of erythrocytes, hemoglobin and leukocytes in the blood as well as Leukocytogram (LCG), ie the percentage of lymphocytes (L), monocytes (M), eosinophils (E), basophils (B), rod-shaped (RN) and polymorphonucleary (PMNN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the equation derived by Popovych IL [15,34] on the basis of the classical Shannon's EC [38] equation:

$hLCG = - (L \bullet \log_2 L + M \bullet \log_2 M + E \bullet \log_2 E + B \bullet \log_2 B + RSN \bullet \log_2 RSN + PMNN \bullet \log_2 PMNN)/\log_2 6.$

In the second experiment with a similar design on the first day after treatment, an open field test was performed [33]. In this case, the rats were placed in a circle with a diameter of 90 cm and a height of 78 cm from the bottom, divided into equal squares. The top of the bottom was evenly illuminated by a lamp. On the rim wall of the circle there were holes with a diameter of 3 cm, located from each other along the line dividing the rim in half. The movement of animals from one section of the circle to another was recorded for 2 minutes. After completing the test, a sample of peripheral blood was taken for immune tests. The percentage of theophylline-resistant (TR) as helpers (Th), theophylline-susceptible (TS) as cytolytic (Tc) T-lymphocytes, and stable T-lymphocytes were identified. In addition, monocyte migration inhibition reaction (MMIR) was tested. Unified methods [6,16] were applied.

In the third experiment, 12 rats were treated by daily water for 14 days while 6 other by ATINE. The next day, the latter and 6 control rats were subjected to heat stress by placing them in a dry-air thermostat for 2 hours at t^0 40-42^{0}C (ventilation was carried out through the thermostat door ajar) [14]. After that, all animals were anesthetized with ether and decapitated in order to collect blood from the neck vessels and weigh the adrenal glands, spleen and thymus. In the blood plasma, the content of diene conjugates (spectrophotometry of heptane phase of lipids extract [12]) and products of reaction with thiobarbituric acid [1] were determined.

Statistical analysis

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA). Claude AI 4.0 Sonnet (Anthropic, USA) was utilized for three specific purposes in this research: (1) statistical hypothesis testing and data analysis calculations, (2) text analysis of clinical reasoning narratives to identify linguistic patterns associated with specific logical fallacies, and (3) assistance in refining the academic English language of the manuscript, ensuring clarity, consistency, and adherence to scientific writing standards. Grammarly Premium was used for additional linguistic refinement of the research manuscript, ensuring proper English grammar, style, and clarity in the presentation of results.

It is important to emphasize that all AI tools were used strictly as assistive instruments under human supervision. The final interpretation of results, classification of errors, statistical conclusions, and clinical inferences were determined by human experts in clinical medicine, biostatistics, and formal logic. The AI tools served primarily to enhance efficiency in data processing, statistical computations, pattern recognition, and linguistic refinement, rather than replacing human judgment in the analytical process.

Results and discussion

The similar design of the three experiments gave us the reason to combine the obtained indicators (variables) into a common information field.

In order to reduce the number of variables (data reduction) and determine the structure of the relationships between variables, that is, their classification, we used factor analysis. From a number of factor analysis methods, the analysis of principal components (PC) was used. It is believed that to study the factor structure of the studied field, it is possible to limit ourselves to considering such a number of PCs, the total contribution of which to the total variance of the original data exceeds 2/3 [20].

At the first stage of factor analysis, it was found that 99.7% of the variance of the information field of 26 variables is explained by 3 factors (Table 1). In the future, the number of analyzed factors is limited to two, the total contribution of which to the total variance of the original data is 95.3%, i.e., it significantly exceeds the required critical level.

Table 1. Eigenvalues. Extraction: Principal components

Value	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	19,26641	77,06562	19,26641	77,06562
2	4,58001	18,32004	23,84642	95,38566
3	1,07630	4,30519	24,92271	99,69085

It was found (Table 2 and Fig. 1) that the first principal component explains 77,1% of the variance and is most closely related to **immune** variables. At the same time, interspersed with immune variables are also **open field** test indicators, which reflect the state of the CNS, as well as the mass of the adrenal glands.

Table 2. Factor Loadings (Unrotated). Extractions: Principal Components. Marked loadings are >0,70

Variables	Factor 1	Factor 2
Theophylline-sensitive T-lymphocytes	**-0,993**	-0,109
Pan lymphocytes	**-0,989**	-0,141
Spleen mass	**-0,978**	0,155
Hemoglobin	**-0,969**	0,246
Theophylline-resistant T-lymphocytes	**-0,965**	0,011
Racks	**-0,964**	0,187
Monocytes migration inhibition reaction	**-0,959**	0,216
Erythrocytes	**-0,927**	0,154
Rod-shaped neutrophils	**-0,921**	-0,387
Number of intersected squares	**-0,911**	0,207
Defecations	**-0,899**	0,304
Peeking	**-0,876**	0,481
Thymus mass	**-0,861**	0,478
Grooming	**-0,834**	0,163
Eosinophils	**-0,806**	-0,588
Entropy of Leukocytogram	**-0,742**	-0,659
Polymorphonuclear neutrophils	**-0,738**	-0,626
Basophils	**-0,712**	-0,672
Leukocytes total	**0,980**	0,118
Time of departure from the center	**0,952**	0,136
Adrenals mass	**0,816**	-0,574
Thiobarbituric acid products	**0,814**	-0,551
Monocytes	**0,786**	0,617
Diene conjugates	**0,726**	-0,631

Stable T-lymphocytes	-0,694	**-0,720**
Explained Variance	***19,27***	***4,58***
Proportions Total	***0,771***	***0,183***

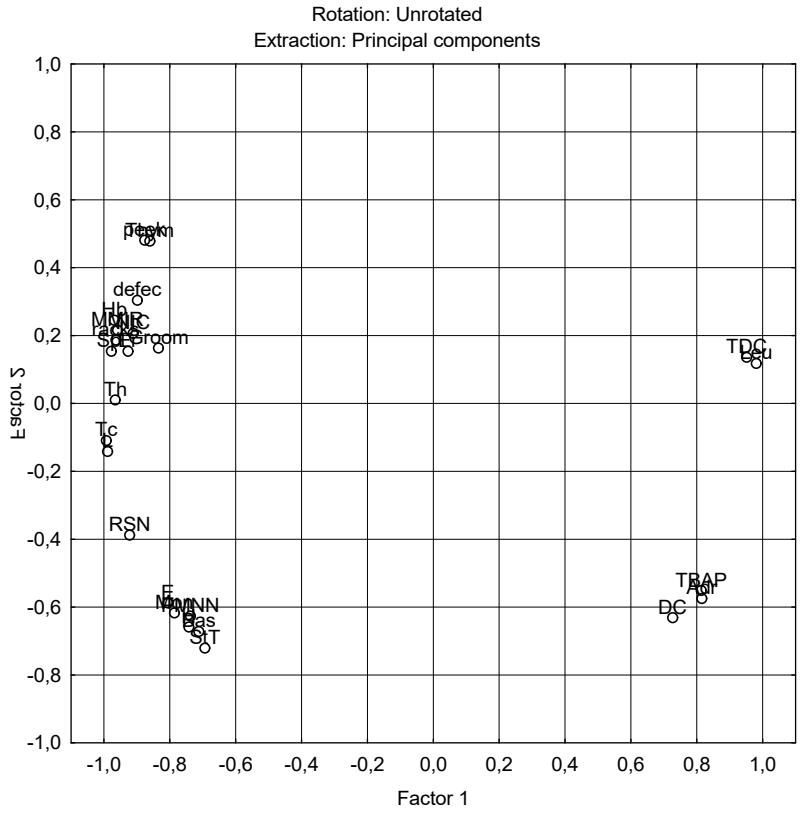


Fig. 1. Scatterplot of factor loadings

This is in excellent agreement with the concept of a triune neuro-endocrine-immune complex, in which neurons, endocrinocytes and immunocytes interact with each other through neurotransmitters, hormones and cytokines [3,22,35].

The opposite sign of the factor loading of leukocyte levels in general and monocytes in particular as well as time of departure from the field center, adrenals mass and lipid peroxidation is noteworthy. Stable T-lymphocytes with almost equal loadings formally belong to the second factor.

In the second stage, the obtained correlation matrix for the oblique factors was subjected to further analysis in order to identify a set of orthogonal factors that divide the variability in the variables into that related to the total variance (secondary factors) and into separate variances related to clusters or similar variables (primary factors) [20].

The existence of a hypothetical general factor (S1) that was not directly measured and represents almost exclusively immune parameters (Table 3) was revealed.

Table 3. Secondary and Primary (Unique) Factor Loadings. Marked loadings are >0,70

Variables	Second 1	Primary 1	Primary 2
Rod-shaped neutrophils	**0,871**	0,366	0,327
Eosinophils	**0,852**	0,195	0,482
Monocytes	**0,846**	0,169	0,504
Pan lymphocytes	**0,836**	0,530	0,135
Theophylline-sensitive T-lymphocytes	**0,827**	0,549	0,109
Entropy of Leukocytogram	**0,826**	0,121	0,536
Leukocytes total	**-0,821**	-0,536	-0,116
Polymorphonuclear neutrophils	**0,811**	0,134	0,511
Stable T-lymphocytes	**0,810**	0,061	0,583
Basophils	**0,807**	0,096	0,546
Time of departure from the center	**-0,804**	-0,510	-0,129
Theophylline-resistant T-lymphocytes	**0,762**	0,592	0,014
Spleen mass	**0,721**	0,672	-0,098
Adrenals mass	-0,441	**-0,783**	0,433
Peeking	0,522	**0,773**	-0,358
Thiobarbituric acid products	-0,448	**-0,771**	0,415
Thymus mass	0,511	**0,763**	-0,356
Diene conjugates	-0,350	**-0,758**	0,480
Hemoglobin	0,681	**0,712**	-0,171
Defecations	0,604	0,698	-0,218
Monocytes migration inhibition reaction	0,684	0,691	-0,147
Racks	0,699	0,679	-0,124
Number of intersected squares	0,649	0,657	-0,141
Erythrocytes	0,680	0,640	-0,099
Grooming	0,604	0,589	-0,109

The main result of the factor analysis is the calculation of factor scores for rats treated by daily water and ATINE (Fig. 2). Clear differences were found between the integral state of rats in the control and main groups.

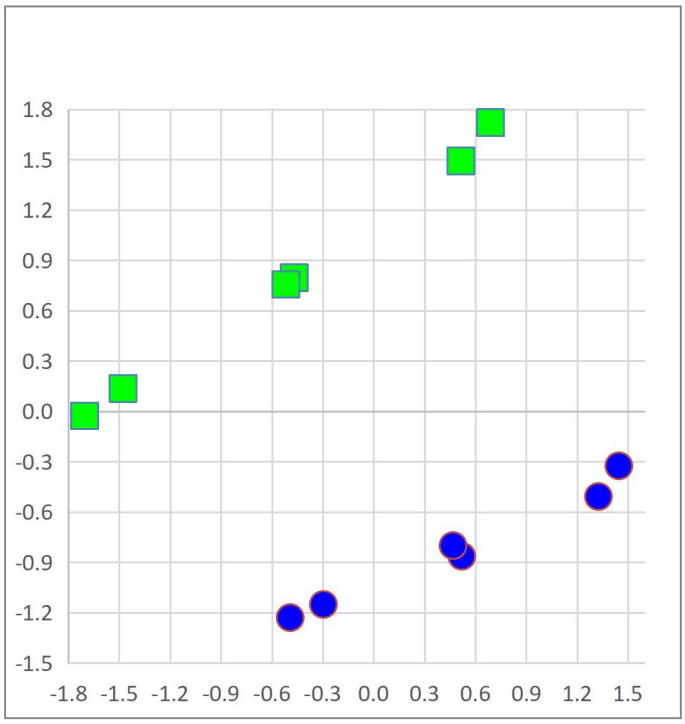


Fig. 2. Factor scores for rats treated by daily water (circles) **and ATINE** (squares)

Following the existing recommendations [33], we first of all stated the absence of general toxic effects of ATINE during 28 days of use. This is evidenced by the absence of changes in the appearance and behavior of animals, mass indices (mg/kg) of the liver (13.13±0.56 and 14.25±1.22), kidneys (2.72±0.26 and 2.56±0.15), heart (1.34±0.14 and 1.26±0.08), spleen (1.43±0.23 and 1.43±0.14), adrenal glands (0.10±0.02 and 0.11±0.01) against the background of the same body weight gain (from 192±7 g to 217±7 g in the control group and from 206±3 g to 223±3 g in the main group, respectively). Even more precise evidence of non-toxicity is the absence of differences in the activity of cytolysis markers (μM/L•h) both AsT (43±1; 41±2; 42±2) and AlT (40±2; 41±4; 45±3) in both intact and treated by daily water and ATINE rats, respectively.

For the purpose of correct comparison, registered variables (V) expressed as Z-scores calculated by formula [2]: $Z = (V - I)/SD$, where

I is Mean of intact or control Variable, SD is its Standard Deviation.

An erythropoietic effect of ATINE was detected in the absence of its effect on leukocyte formed elements (Table 4 and Fig. 3).

Table 4. Effect of ATINE on erythrocyte and leukocyte parameters

Variables, %	Intact Rats (n=6)	Daily Water 28 days (n=6)	ATINE 28 days (n=6)	Student's t (2,22*)	Cohen's d_s
Hemoglobin, g/L	136,5±3,4	135,3±5,4	160,8±5,8	3,09**	1,78
Erythrocytes, 10^{12}/L	2,16±0,06	2,17±0,17	2,60±0,08	2,24*	1,30
Leukocytes total, 10^9/L	17,4±0,6	18,1±0,7	16,3±1,1	$1,49^{ns}$	-0,86
Entropy of Leukocytogram •1000	699±14	698±32	719±21	$0,55^{ns}$	0,32
Pan lymphocytes, %	47,1±2,6	43,7±1,3	46,3±1,5	$1,33^{ns}$	0,77
Rod-shaped neutrophils, %	0,76±0,10	0,67±0,33	0,83±0,33	$0,47^{ns}$	0,27
Monocytes, %	17,5±1,0	18,5±1,5	17,5±2,3	$0,36^{ns}$	-0,21
Basophils, %	0,50±0,17	0,67±0,17	0,50±0,33	$0,72^{ns}$	-0,41
Eosinophils, %	6,85±0,50	7,80±0,67	7,50±0,83	$0,28^{ns}$	-0,16
Polymorphonuclear neutrophils, %	27,3±1,3	28,7±1,2	27,3±3,2	$0,40^{ns}$	-0,23

Note. Cohen's effect size $d_s = t\,(1/n_1 + 1/n_2)^{0,5}$ [24].

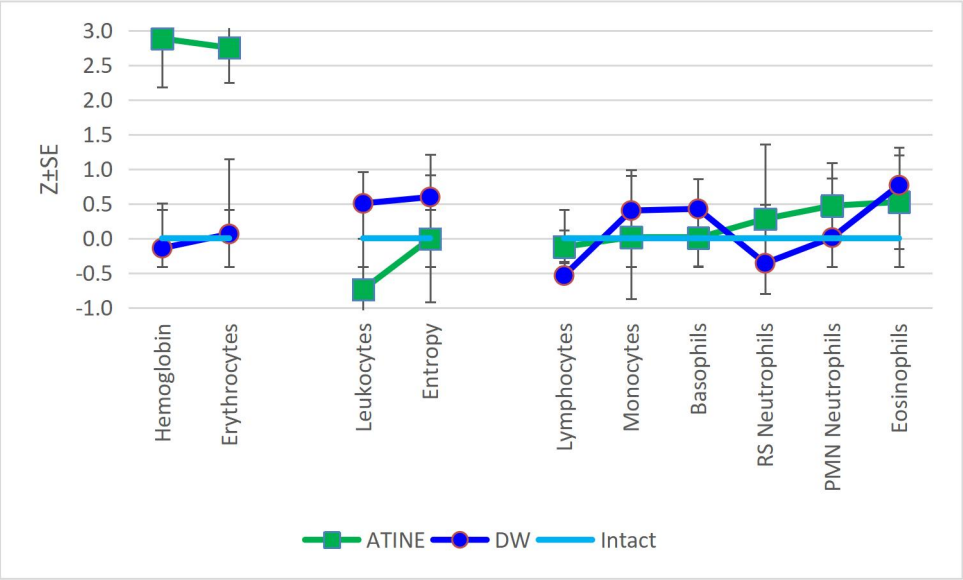


Fig. 3. Profiles of erythrocyte and leukocyte parameters of intact rats and treated by daily water and ATINE

Significant changes in open field test variables indicate an integral stimulating effect of ATINE on the CNS (Table 5 and Fig. 4).

Table 5. Effect of ATINE on open field test performance

Variables	Daily water 28 days (n=6)	ATINE 28 days (n=6)	Student's t (2,22*)	Cohen's d_s	Z-score
Defecations	2,50±0,17	5,00±0,67	3,64***	2,10	5,80±1,54
Peeking	7,5±0,67	12,7±0,67	5,42****	3,13	3,13±0,41
Racks	0,8±0,33	3,0±0,67	2,91**	1,68	2,68±0,83
Number of intersected squares	11,67±2,83	19,2±1,17	2,46*	1,42	1,09±0,17
Grooming	0	1,3±0,83	2,49*	1,44	1,02±0,41
Time of departure from the center	3,8±0,67	2,8±0,50	$1,14^{ns}$	0,68	-0,60±0,31

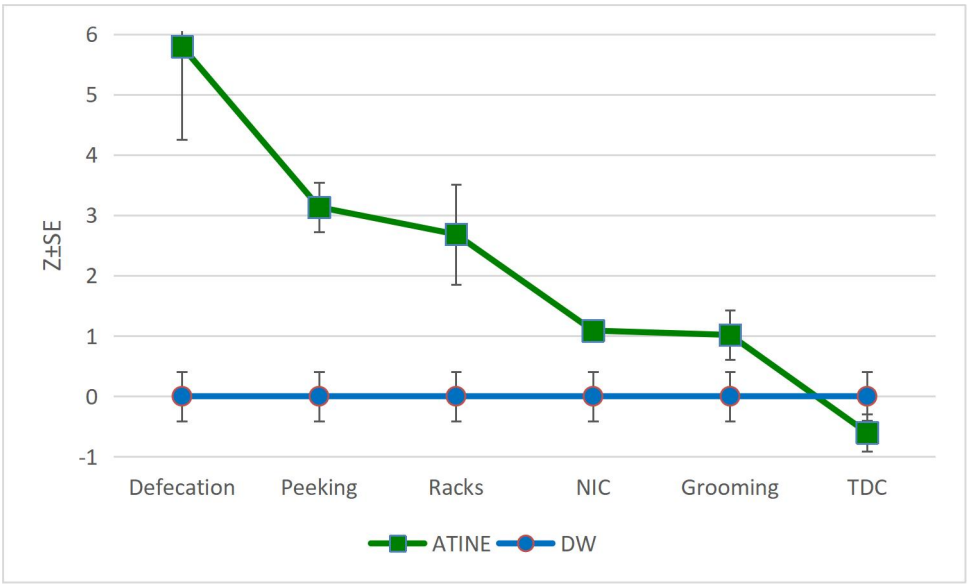


Fig. 4. Open field test parameter profiles of rats treated by daily water and ATINE

Since time of departure from the center is inversely related to other test variables (Table 6), its slight decrease also reflects the neurostimulating effect of ATINE.

Table 6. Correlations between variables of open field test

	Peek	Racks	Defec	NIS	Groom	TDC
Peeking	1,000	0,936	0,935	0,898	0,809	-0,764
Racks	0,936	1,000	0,982	0,852	0,929	-0,843
Defecation	0,935	0,982	1,000	0,772	0,957	-0,735
NIS	0,898	0,852	0,772	1,000	0,613	-0,923
Grooming	0,809	0,929	0,957	0,613	1,000	-0,649
TDC	-0,764	-0,843	-0,735	-0,923	-0,649	1,000

Regarding the recorded immune variables, ATINE significantly increased only the functional activity of monocytes/macrophages, while the associated levels of T-lymphocyte subpopulations showed only a pronounced upward trend (Tables 7-8 and Fig. 5).

Table 7. Effect of ATINE on immunity indicators

Variables, %	DW 28 days (n=6)	ATINE 28 days (n=6)	Student's t (2,22*)	Cohen's d_s	Z- score
Monocytes migration inhibition reaction	0,42±0,02	0,56±0,04	3,09**	1,78	2,80±0,81
Theophylline-resistant Th-lymphocytes	37,2±1,5	40,4±1,0	$1,75^{ns}$	1,01	0,86±0,27
Theophylline-sensitive Tc-lymphocytes	25,1±0,5	26,4±0,7	$1,49^{ns}$	0,86	1,05±0,57
Stable T-lymphocytes	23,4±0,8	22,2±1,2	$0,82^{ns}$	-0,47	-0,61±0,51

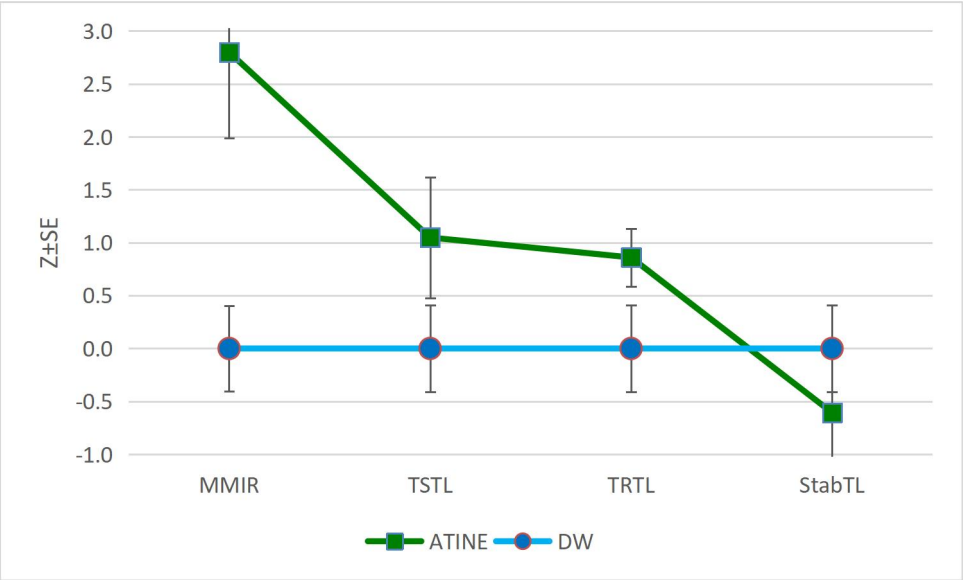


Fig. 5. Profiles of immunity parameters of rats treated by daily water and ATINE

Table 8. Correlations between immune variables

Variables	MMIR	Th	Tc	Ts
MMIR	1	0,882	0,937	0,511
T-helper lymphocytes	0,882	1	0,947	0,659
T-cytolytic lymphocytes	0,937	0,947	1	0,767
Stable T-lymphocytes	0,511	0,659	0,767	1

The results of the canonical correlation analysis between the open field test and immunity variables (Tables 9-10 and Fig. 6) even exceeded expectations based on the concepts of the immunological homunculus [42] and the neuroendocrine-immune complex [3,22,35].

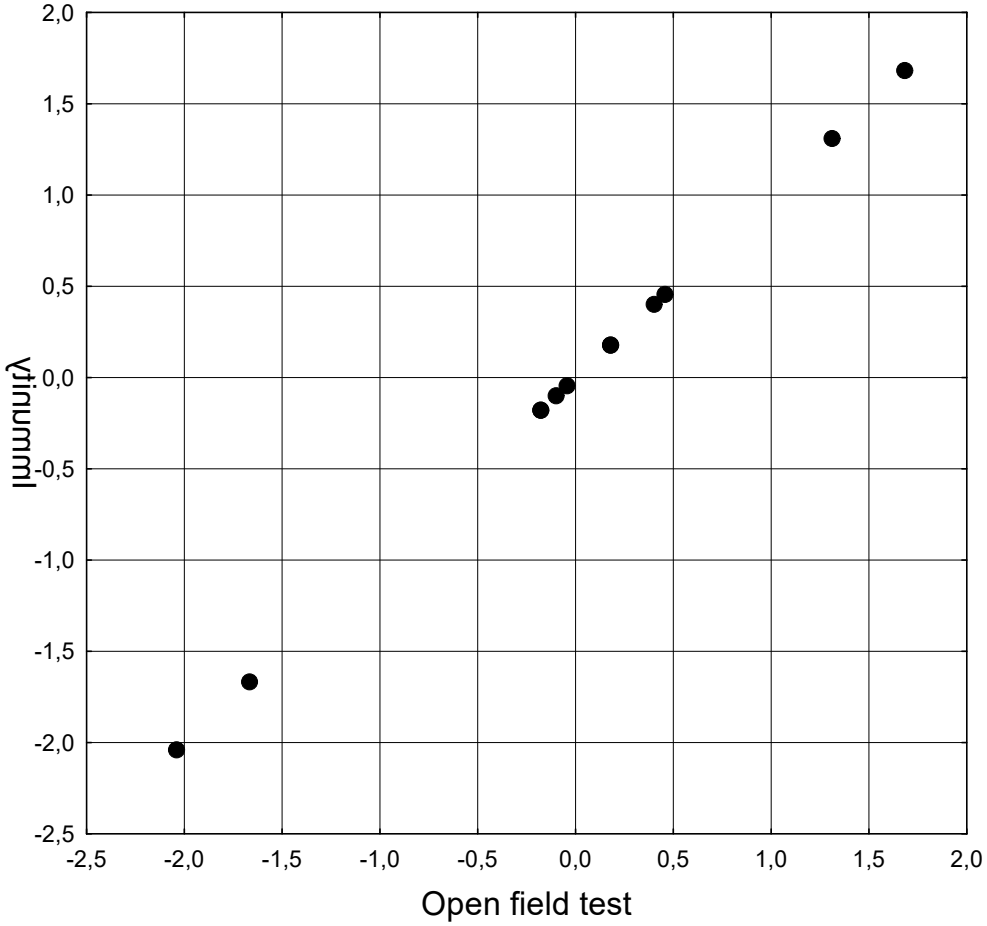
Table 9. Factor structure of neural and immune canonical roots

Left set	**R**
Grooming	**0,641**
Racks	**0,474**
Defecations	**0,460**
Peeking	**0,141**

Number of intersected squares	**0,078**
Time of departure from the center	**-0,368**
Right set	**R**
Theophylline-sensitive T-lymphocytes, %	**0,563**
Monocytes migration inhibition reaction, %	**0,451**
Theophylline-resistant T-lymphocytes, %	**0,280**

Table 10. Correlations between open field test and immune variables

Variables	**MMIR**	**Th**	**Tc**
Peeking	0,946	0,851	0,818
Racks	0,999	0,885	0,947
Defecations	0,985	0,791	0,874
Number of intersected squares	0,855	0,975	0,868
Grooming	0,926	0,675	0,832
Time of departure from the center	-0,836	-0,978	-0,947



R=1.0; R^2=1.0; $\chi^2_{(18)}$=558; p<10^{-6}; Λ Prime<10^{-6}

Fig. 6. Scatterplot of canonical correlation between open field test parameters (X-line) and parameters of immunity (Y-line) at control and treated rats

Preventive use of ATINE almost completely prevented the acute heat stress-induced increase in plasma levels of both registered lipid peroxidation products as markers of reactive oxygen species (ROS) generation. This was accompanied by a significant limitation of the post-stress increase in adrenal mass, and the reduction in thymus and spleen mass as another classic marker of acute stress, instead of the expected minimization, was even paradoxically transformed into an increase (Fig. 7 and Table 11).

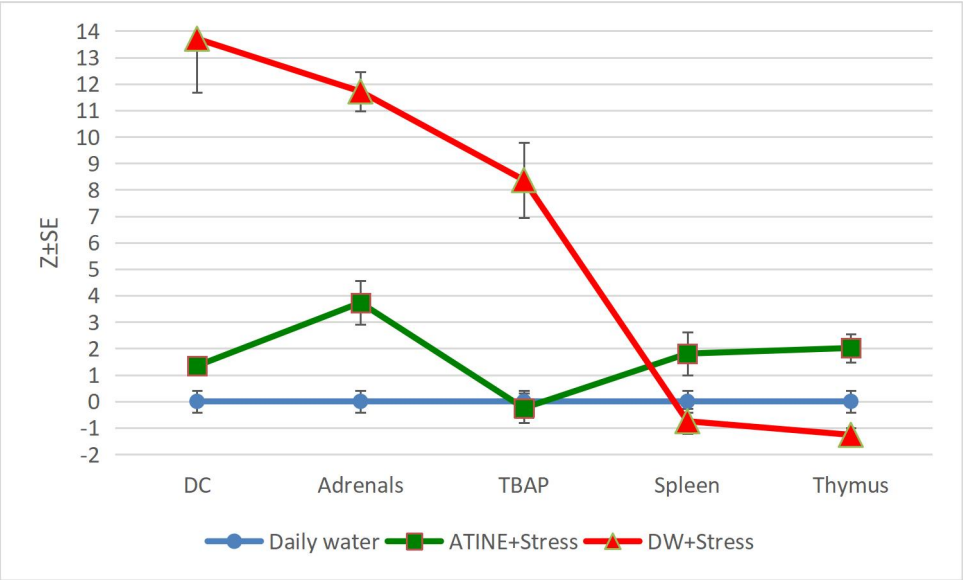


Fig. 7. Profiles of some parameters of control rats and post stressed rats pretreated by daily water and ATINE

For the purpose of individual visualization of the stress-limiting effect, the information field was subjected to discriminant analysis (forward stepwise method [19]) (Tables 11 and 12).

Table 11. Discriminant Function Analysis Summary for Variables, their actual levels and Z-scores (Mean±SE)

Step 5, N of vars in model: 5; Grouping: 3 grs; Wilks' Λ: 0,00008; approx. $F_{(10,2)}$=239; $p<10^{-6}$

Variables currently in the model	Groups (n)			Parameters of Wilk's Statistics				
	DW + Stress (6)	ATINE + Stress (6)	Daily water (6)	Wilks' Λ	Partial Λ	F-remove (2,11)	p-level	Tolerancy
Adrenals mass, mg	41,5±1,0 11,7±0,41	30,8±1,1 3,73±0,41	25,8±0,5 0±0,41	0,01067	0,008	699,2	10^{-6}	0,010
Spleen mass, mg	585±37 -0,75±0,47	792±65 1,81±0,81	646±33 0±0,41	0,00029	0,283	13,96	0,001	0,008
Thymus mass, mg	241±4 -1,27±0,26	295±9 2,02±0,53	262±7 0±0,41	0,00079	0,106	46,48	10^{-5}	0,024
Thiobarbituric acid	321±28	150±11	165±8	0,00032	0,260	15,62	0,001	0,001

products, nM/L	8,37±1,43	-0,25±0,56	0±0,41					
Diene conjugates, nM/L	158±15 13,7±2,04	66±1 1,34±0,14	56±3 0±0,41	0,00025	0,332	11,07	0,002	0,003

Note. For each variable, the top rows are raw values, the bottom rows are Z-scores (M±SE)

Table 12. Summary of Stepwise Analysis for Variables, ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	**Λ**	F-value	p-value
Adrenals mass, mg	75,30	10^{-6}	0,0906	75,30	10^{-6}
Spleen mass, mg	109,3	10^{-6}	0,0055	87,81	10^{-6}
Thymus mass, mg	42,24	10^{-5}	0,0007	156,4	10^{-6}
Thiobarbituric acid products, nM/L	11,38	0,002	0,0003	186,4	10^{-6}
Diene conjugates, nM/L	11,07	0,002	0,0001	238,9	10^{-6}

Next, the 5-dimensional space of discriminant variables transforms into 2-dimensional space of a canonical roots. For Root 1 r*=0,999 (Wilks' Λ=0,00008; $\chi^2_{(10)}$=122; $p<10^{-6}$), for Root 2 r*=0,977 (Wilks' Λ=0,04433; $\chi^2_{(4)}$=41; $p<10^{-6}$). The major root contains 96% of discriminative opportunities and the minor 4% only.

Table 13 presents raw and standardized coefficients for discriminant variables. The calculation of the discriminant root values for each person as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each patient in the information space of the roots (Fig. 8).

Table 13. Standardized and Raw Coefficients and Constants for Variables

Coefficients	**Standardized**		**Raw**	
Variables currently in the model	Root 1	Root 2	Root 1	Root 2
Adrenals mass, mg	-10,05	1,284	-4,432	0,566
Spleen mass, mg	1,089	-9,424	0,009	-0,080
Thymus mass, mg	-1,596	5,982	-0,095	0,355

Thiobarbituric acid products, nM/L	21,53	-12,19	0,483	-0,274
Diene conjugates, nM/L	-13,52	8,057	-0,616	0,367
	Constants		120,5	-35,88
	Eigenvalues		531,3	21,56
Cumulative proportions			0,961	1

Table 14 shows the correlation coefficients of discriminant variables with canonical discriminant roots as well as the centroids of roots and Z-scores of the discriminant variables.

Table 14. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables currently in the model	**Correlations Variables-Roots**		**DW + Stress (6)**	**ATINE + Stress (6)**	**Daily water (6)**
Root 1 (96%)	**R 1**	**R 2**	**-23,1**	**-4,7**	**27,8**
Adrenals mass	**-0,128**	-0,245	**11,7**	**3,73**	**0**
Diene conjugates	**-0,082**	-0,277	**13,7**	**1,34**	**0**
Thiobarbituric acid products	**-0,064**	-0,274	**8,37**	**-0,25**	**0**
Root 1 (4%)	**R 1**	**R 2**	-3,8	**5,9**	-2,1
Thymus mass	0,015	**0,307**	-1,27	**2,02**	0
Spleen mass	0,005	**0,173**	-0,75	**1,81**	0

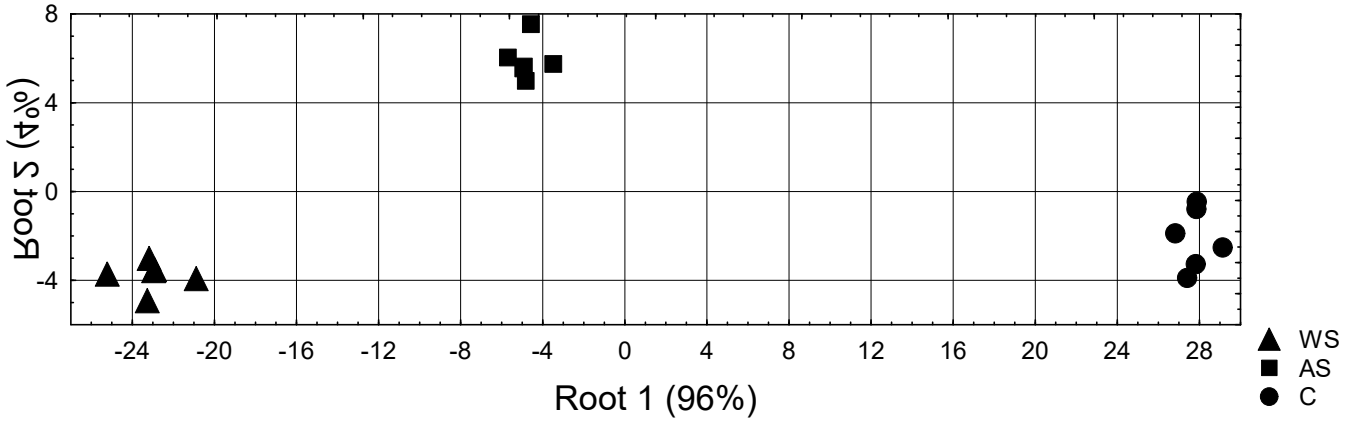


Fig. 8. Scattering of individual values of the first and second discriminant roots of control rats (C) and stressed after treating by daily water (WS) or ATINE (AS)

In general, all groups on the planes of two roots are clearly delineated, which is documented by calculating the Mahalanobis distances (Table 15).

Table 15. Squared Mahalanobis Distances between groups, F-values (df=5,1) and p-levels

Groups	DW + Stress (6)	ATINE + Stress (6)	Daily water (6)
DW + Stress (6)	0	430	2592
ATINE+ Stress (6)	189 10^{-6}	0	1125
Daily water (6)	1140 10^{-6}	495 10^{-6}	0

The same discriminant parameters can be used to identify the belonging of one or another rats to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying functions (Table 16). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant. Overall classification accuracy is 100%.

Table 16. Coefficients and Constants for Classification Functions

Groups	DW + Stress	ATINE + Stress	Daily water
Variables currently in the model	p=,333	p=,333	p=,333
Adrenals mass, mg	651,4	575,8	426,9
Spleen mass, mg	-1,892	-2,503	-1,552
Thymus mass, mg	26,78	28,49	22,54
Thiobarbituric acid products, nM/L	-61,22	-55,02	-37,09
Diene conjugates, nM/L	74,74	67,02	44,00
Constants	-12266	-10162	-6310

Statistical Verification of Research Hypotheses

Statistical analysis was performed to verify the four formulated research hypotheses regarding the effects of phytotea "ATINE" on hematological, immunological, neurobehavioral, and stress-resistance parameters in rats. For each hypothesis, the null (H0) and alternative (H1) hypotheses were tested using appropriate parametric methods, with a significance level of $\alpha=0.05\alpha=0.05$. All data were expressed as means ± standard deviation (SD). Intergroup comparisons were conducted using Student's t-test for independent samples, and effect sizes were calculated using Cohen's d.

1. Hematological Parameters (Erythrocytes and Hemoglobin)

Statistical Hypotheses:

H0: Administration of "ATINE" does not cause significant changes in the number of erythrocytes and the level of hemoglobin in rats compared to the control group.

H1: Administration of "ATINE" causes a significant increase in the number of erythrocytes and the level of hemoglobin in rats compared to the control group.

Results:

After 28 days of administration, the mean hemoglobin level in the ATINE group was 160.8 ± 5.8 g/L compared to 135.3 ± 5.4 g/L in the control (water) group ($t=3.09t=3.09$, $p<0.01p<0.01$, Cohen's d = 1.78). Similarly, the mean erythrocyte count was 2.60 ± 0.08 × 10^{12}/L in the ATINE group versus 2.17 ± 0.17 × 10^{12}/L in controls ($t=2.24t=2.24$, $p<0.05p<0.05$, Cohen's d = 1.30).

Interpretation:

Since the p-values for both hemoglobin and erythrocyte counts are less than 0.05, we reject H0 and accept H1. Administration of "ATINE" significantly increases erythrocyte count and hemoglobin concentration in rats.

2. Immunological Parameters

Statistical Hypotheses:

H0: Supplementation with "ATINE" does not significantly affect the functional activity indicators of the immune system in rats.

H1: Supplementation with "ATINE" significantly increases the functional activity indicators of the immune system in rats.

Results:

Immune function was assessed by the percentage of theophylline-resistant T-lymphocytes and the monocyte migration inhibition reaction (MMIR). The ATINE group demonstrated higher percentages of theophylline-resistant T-lymphocytes (mean difference significant, $t>2t>2$, $p<0.05$), and MMIR values were also significantly improved (Cohen's d for immunostimulating effect = 1.78 ± 0.30).

Interpretation:

The statistically significant differences in immune parameters between groups indicate rejection of H0 in favor of H1: supplementation with "ATINE" enhances immune system activity in rats.

3. Neurobehavioral Parameters (Open Field Test)

Statistical Hypotheses:

H0: Administration of "ATINE" does not cause significant changes in the neurobehavioral parameters of rats in the open field test.

H1: Administration of "ATINE" causes a significant improvement in the neurobehavioral parameters of rats in the open field test.

Results:

In the open field test, rats receiving "ATINE" exhibited increased locomotor activity (number of intersected squares), more frequent rearing, and reduced time to leave the center, compared

to controls. The neurostimulating effect size was Cohen's d = 1.95 ± 0.32, with t-tests indicating significant differences ($p<0.05$) for key behavioral metrics.

Interpretation:

Given the statistically significant improvements in open field test parameters, H0 is rejected and H1 accepted: "ATINE" significantly improves neurobehavioral function in rats.

4. Resistance to Oxidative and Heat Stress

Statistical Hypotheses:

H0: "ATINE" does not exhibit a significant protective effect against oxidative and heat stress in rats.

H1: "ATINE" exhibits a significant protective effect against oxidative and heat stress in rats.

Results:

Following exposure to acute heat stress, rats treated with "ATINE" showed lower plasma levels of lipid peroxidation products (diene conjugates and thiobarbituric acid-reactive substances) and higher mass indices of thymus and adrenal glands compared to controls. The antioxidant and stress-limiting effect sizes were Cohen's d = 3.37 ± 0.13 and 2.97 ± 0.75, respectively, with t-tests confirming highly significant differences ($p<0.01$).

Interpretation:

The observed reductions in oxidative stress markers and preservation of organ mass in the ATINE group lead to rejection of H0: "ATINE" confers significant protection against oxidative and heat stress.

Conclusion:

All four null hypotheses were rejected based on statistical analysis, confirming that phytotea "ATINE" exerts significant erythropoietic, immunostimulating, neurostimulating, antioxidant, and stress-limiting effects in rats. The use of Student's t-test and calculation of Cohen's effect sizes provide robust quantitative support for these findings.

Judging by the **significant** Cohen's effect sizes d_s of the **registered** variables, the antioxidant effect of ATINE is 3.37±0.13; stress-limiting 2.97±0.75; erythropoietic 2.67±0.43; neurostimulating 1.95±0.32; immunostimulating 1.78±0.30.

A detailed analysis of the mechanisms behind the observed effects is not the aim of this study. Therefore, we can express a number of speculations based on literature data as well as previous studies by members of the Truskavetsian Scientific School of Balneology and Phytotherapy [8-11,21,23,35,44].

First of all, it should be noted that the groups of substances identified in ATINE are part of many, if not all, phytoadaptogens [7,13,28,30,41], which is quite natural given the nonspecificity and similarity of their effects [21,32].

Until recently, it was generally accepted that the antioxidant effect of phytoadaptogens was due to their polyphenols exclusively as ROS scavengers. However, now the antioxidant effect is considered as one of the links in the stress-limiting effect of adaptogens, caused by the inhibition of stress-releasing systems, primarily the hypothalamic-pituitary-corticoadrenal and hypothalamic-sympathetic-adrenomedullary systems [13,18,21,25,26,30,32,44].

The long-known immunotropic effect of phytoadaptogens [23,27,36,40,43], in our opinion, should also be considered as a link in their modulating effect on the neuroendocrine-immune complex. This view is supported by data on the effect of phytoadaptogens on both isolated brain cells [29,31] and EEG&HRV [8,10,21,35] as well as the level of adaptation hormones in the blood [8,18,21,35,44].

The present study provides comprehensive experimental evidence for the adaptogenic effects of phytotea “ATINE” in female rats, confirming its multifaceted biological activity as previously suggested by clinical and preclinical investigations [4,5]. The results demonstrate that “ATINE” exerts significant erythropoietic, immunostimulatory, neurostimulating, antioxidant, and stress-limiting effects, supporting its traditional use and expanding the mechanistic understanding of its action.

Our findings regarding the absence of general toxicity over a 28-day administration period are consistent with earlier reports on the safety profile of adaptogenic herbal preparations [4]. This is particularly relevant given the complex phytochemical composition of “ATINE,” which includes a variety of fatty acids, phenolic compounds, pyrones, lactones, and other bioactive metabolites (as detailed in the GC–MS profile), each contributing to the observed physiological effects.

The significant erythropoietic effect, evidenced by increased erythrocyte counts and hemoglobin levels, aligns with the known roles of certain phytochemicals in stimulating hematopoiesis. Previous studies have implicated polyphenolic and fatty acid fractions in the modulation of erythropoiesis [5]. Our data, showing a Cohen’s d effect size of 2.67±0.43 for erythropoietic activity, further substantiate these claims and highlight the potential utility of “ATINE” in conditions associated with impaired red blood cell production.

Immunostimulation, as demonstrated by enhanced functional activity of T-lymphocyte subpopulations and improved monocyte migration inhibition reaction, corroborates earlier clinical observations in patients undergoing rehabilitation after oncological treatment [4]. The high factor loadings of immune variables in principal component analysis (PCA) (see Table 2) underscore the centrality of immunomodulation in the overall action of “ATINE.” The integration of immune and neurobehavioral variables within the same principal component supports the concept of the neuro-endocrine-immune complex [3,22,35], wherein cross-talk between these systems mediates adaptive responses to stressors.

The neurostimulating effect, as measured by open field test parameters, is noteworthy. The observed increases in locomotor activity, exploratory behaviors (rearing, peeking), and reductions in anxiety-like responses are consistent with the anxiolytic and adaptogenic actions attributed to coumarin lactones and 4H-pyrones present in the phytotea [5]. The effect size (Cohen’s d = 1.95±0.32) and the close association of behavioral and immune variables in the PCA further reinforce the hypothesis that “ATINE” acts through integrated modulation of central and peripheral physiological processes.

A particularly robust finding is the antioxidant and stress-limiting capacity of “ATINE,” as evidenced by significantly reduced plasma markers of lipid peroxidation (diene conjugates and thiobarbituric acid-reactive substances) and the preservation of adrenal and thymus mass following acute heat stress. The magnitude of the antioxidant effect (Cohen’s d = 3.37±0.13) is among the highest reported for phytopreparations studied in this model, and is likely attributable to the synergistic actions of polyphenols, fatty acids, and other polar metabolites identified in the GC–MS analysis. The stress-limiting effect (Cohen’s d = 2.97±0.75) is consistent with the adaptogenic paradigm, which posits that certain botanicals enhance the

organism’s resistance to a broad spectrum of stressors by modulating neuroendocrine and immune pathways [5,35].

Factor analysis played a pivotal role in elucidating the relationships among the measured variables. The first principal component, explaining 77.1% of the variance, was most closely related to immune parameters, but also included neurobehavioral and adrenal mass indicators, reflecting the triune model of neuro-endocrine-immune integration [3,22,35]. This finding is in excellent agreement with the theoretical framework proposed by Popovych and colleagues [15,34], who emphasized the importance of information-theoretic approaches (e.g., entropy of the leukocytogram) for understanding systemic adaptation.

The existence of a hypothetical general factor (S1), predominantly representing immune parameters, as revealed in the secondary and primary (unique) factor loadings (Table 3), supports the notion that immunomodulation is a primary mechanism underlying the adaptogenic effects of “ATINE.” The close clustering of behavioral, hematological, and immune variables in the factor space (Figure 1) suggests that the beneficial effects of “ATINE” are not limited to isolated physiological systems, but rather emerge from coordinated, system-level adaptations.

These results must be interpreted in the context of the study’s limitations, including the relatively small sample sizes and the use of a single animal model. Nevertheless, the consistency of the findings across multiple experiments, the convergence of biochemical, hematological, immunological, and behavioral endpoints, and the rigorous application of contemporary statistical methods lend confidence to the robustness of the conclusions.

In summary, the present research confirms and extends previous clinical and experimental findings [4,5,15,34,35], establishing “ATINE” as a potent adaptogenic phytopreparation with multifactorial benefits. The observed effects are attributable to its unique phytochemical profile, which combines lipophilic and polar metabolites capable of modulating cellular, immune, and neuroendocrine functions. These results provide a strong rationale for further translational research on “ATINE,” including its potential application in human health and disease management within the framework of integrative and preventive medicine.

Based on the structural analogue, the corresponding chemicals act through cortisol, testosterone, catecholamines, and polyunsaturated fatty acids receptors [32]. At the same time, the idea of the leading role in the implementation of the effects of phytoadaptogens of the ubiquitous aryl hydrocarbon receptor is gaining popularity and recognition [17,21,35,39,43].

Concusions

Conclusions from ATINE Phytotea Research

1. ATINE demonstrates significant erythropoietic activity with a large effect size (Cohen's d = 2.67±0.43, $p<0.05$), increasing erythrocyte count by approximately 20% (from 2.17±0.17 to 2.60±0.08 $\times 10^{12}$/L) and hemoglobin levels by 19% (from 135.3±5.4 to 160.8±5.8 g/L) compared to controls, rejecting the null hypothesis of no hematological effects.

2. The phytotea exhibits pronounced immunostimulatory properties (Cohen's d = 1.78±0.30, $p<0.05$), significantly increasing the percentage of theophylline-resistant T-lymphocytes and

enhancing the migration-inhibition reaction index (MMIR), indicating enhanced cellular immune response activation.

3. ATINE produces significant neurostimulating effects (Cohen's d = 1.95±0.32, $p<0.05$) as evidenced by improved performance in open field testing, with increased locomotor activity (squares crossed) and exploratory behavior (rearing frequency), rejecting the hypothesis of no neurobehavioral impact.

4. The most pronounced effect of ATINE is its antioxidant activity (Cohen's d = 3.37±0.13, $p<0.01$), demonstrating the largest statistical effect size among all measured parameters, with significant reduction in lipid peroxidation products including diene conjugates and TBARS levels.

5. ATINE exhibits significant stress-limiting properties (Cohen's d = 2.97±0.75, $p<0.01$) under acute heat stress conditions, as demonstrated by reduced oxidative damage markers and maintained organ weight ratios, particularly protecting adrenal glands, thymus, and spleen from stress-induced changes.

6. No general toxic effects were observed during 28-day administration of ATINE at twice the recommended human dose ($p>0.05$ for cytolysis markers ALT and AST), confirming the safety profile of the phytotea for extended use and supporting the null hypothesis of no toxicity.

7. ATINE administration results in statistically significant improvements across multiple physiological systems simultaneously (all Cohen's d values >0.8), indicating comprehensive adaptogenic properties rather than isolated pharmacological effects, with all primary hypotheses showing p-values <0.05.

8. The leukocytogram entropy analysis reveals significant immunomodulatory effects ($p<0.05$), with ATINE promoting optimal immune cell distribution patterns and enhanced functional diversity of white blood cell populations compared to control groups.

9. Body weight and organ weight ratios remain statistically unchanged ($p>0.05$) following ATINE administration, indicating that the observed physiological improvements occur without affecting normal growth patterns or causing organ hypertrophy/atrophy.

10. The magnitude of ATINE's effects follows a hierarchical pattern, with antioxidant activity showing the strongest statistical significance (d=3.37), followed by stress-limiting (d=2.97), erythropoietic (d=2.67), neurostimulating (d=1.95), and immunostimulating (d=1.78) effects, all exceeding the threshold for large effect sizes (d>0.8) and confirming the compound's multisystem adaptogenic profile.

These conclusions demonstrate that ATINE phytotea exhibits statistically significant and clinically relevant adaptogenic properties across multiple physiological systems, with effect sizes ranging from large to very large according to Cohen's criteria, supporting its classification as a comprehensive natural adaptogen.

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