

## SAFETY TONIC (ENERGY) BEVERAGES

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**Abstract:** Volume growth of consumption of tonic (energy) beverages determines the necessity to study safety criteria of the components used in their manufacture, having a tonic effect. Relevancy of the study is stipulated by the fact, that for the first time biological safety of prolonged use of the main components of non-alcoholic tonic (energy) beverages is evaluated by identifying biomarkers damage of cell membranes and integral parameters of metabolism, the results of which are applicable to humans. Under conditions *in vivo* the effect of prolonged use of the main components of tonic (energy) beverages - caffeine, taurine and herbal extracts (adaptogens in traditionally recommended quantities) on a model object was analyzed. The study was performed on 150 adult Wistar rats of both sexes (females,  $n = 75$ ; males,  $n = 75$ ). Experimental animals were divided into 5 groups according to the sort of components of tonic (energy) beverages consumed: 0.03% aqueous solution of caffeine and 0.25 % aqueous solution of taurine; ginseng extract; *Rhodiola rosea* extract; herbal extract of *Schizandra chinense*. In the control group the purified bottled water was used. In all groups of animals after three weeks of intake of (energy) beverage components, specific biomarkers of organ-and-tissue damage of cell membranes were determined in serum and tissue: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) and integral basic metabolic parameters: glucose, uric acid, urea and total cholesterol. It was stated, that with prolonged daily use of aqueous solutions of caffeine and taurine in amounts corresponding to the content in tonic (energy) beverages, there was a significant increase in activity LDH and CK ( twice and six times, respectively) concentration of urea and uric acid in serum. There is a tendency to increasing action of AST and hypoglycemia, reflecting subclinical pathogenetically for meaningful increase in metabolic rate with a predominance of catabolic processes. In the usage of herbal extracts as adaptogens metabolism is stimulated with a predominance of catabolic processes, mostly pronounced in the use of extracts of ginseng and lemongrass. However, the metabolic adjustment does not go beyond the range of physiological adaptation – not of a pathogenetically significant increase in the activity of enzymes, or biomarkers damage of cell membranes and hypoglycemia. On the contrary, there is a tendency to the development of hyperglycemia. Some specific gender metabolic reconstructions with prolonged use of restorative components of (energy) beverages are revealed.

**Keywords:** Biological safety, tonic beverages, caffeine, taurine, herbal extracts, biomarkers

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### INTRODUCTION

A distinctive feature of non-alcoholic tonic (energy) beverages is the ability to have a tonic effect on the functional activity of individual organs and tissues of a human body as a whole, the effectiveness of which may vary depending on the physiological state, age and sex.

According to the results of numerous studies, the main consumers of tonic (energy) beverages are men aged 30–35 years. This is largely due to the peculiarities

of life and social and community activities of the male population, in particular the need for more emotional stimulation, overcoming tiredness and stress, as well as compensation for the lack of so-called "healthy" food [1, 2, 3, 4, 5, 6].

Consumption and sales in this segment are growing. According to the results of previous studies that we reviewed, the level of consumption of tonic (energy) beverages in Russia will be increased by 1.5 liters per

capita compared to 2014, and it will be 2.9 liters by 2017. In highly developed countries, such as the USA, Germany, the UK, during the period from 2014 to 2017 level of tonic (energy) beverages consumption will be increased by 10.6 liters, 0.9 liters, 1.5 liters per capita respectively [7].

One of the main components of tonic (energy) beverages is caffeine. Its attractiveness for producers and consumers is stipulated by the availability and quite pronounced stimulating effect on mental and physical abilities of a person, which helps to keep fit during tiredness [8]. After intake of beverages with caffeine tiredness and drowsiness are temporarily eliminated or reduced. Caffeine has a great influence on the highest nervous activity of an organism, which largely depends on the applied dose and component and the type of humans nervous system. When used in small doses it is dominated by the stimulating effect, in large - depressing.

The results of the previous studies to determine presence and duration of tonic effect have confirmed the fact, that caffeinated beverages have a tonic effect lasting up to two or three hours, enhance efficiency and rapid involvement in the process, increase mental and physical performance. However, after drinking tonic (energy) beverages some deterioration of health is possible with the development of unpleasant subjective sensations (knock on his temples, nausea, physical weakness) and increased systolic and diastolic blood pressure, pulse rate [9]. Adverse effects occur with long-term consumption of caffeine-containing beverages in doses greater than 400 mg / day.

Consumption of caffeine-containing beverages is necessary to limit for the elderly and children.

For the elderly the effect on sleep is more pronounced: the coming slows down, the total sleep time is reduced, the frequency of awakenings increases, possibly due to the rapid metabolism of catecholamines of the central nervous system (CNS). Drinking caffeinated beverages can adversely affect the central nervous system.

Children, taking caffeine beverages with high doses of caffeine, may experience negative effects on the cardiovascular, genitourinary, nervous systems, gastrointestinal tract [8].

Another important component of tonic (energy) beverages is taurine, which helps to normalize the function of cell membranes, the optimization of energy and metabolic processes, maintain constant electrolytic composition of the cytoplasm of cells, inhibition of synaptic transmission. Taurine at high doses (greater than 1 g) has a suppressive effect on brains. In small doses, in combination with alcohol, caffeine and other stimulants of taurine containing drinks can cause excitation. Therefore it is not recommended to use taurine containing beverages to people with increased excitability and increased susceptibility to alcohol. One-time reception of taurine in a higher dose can cause a feeling of tiredness.

Of a particular interest are the studies of ginseng components influence on the immune system. Ginseng extract can have exertamodulatory effects on

phagocytic cells and lymphocytes producing antibodies in humans and animals, increase the proliferative response of human lymphocytes in the minimum, inhibit concentrations and have the response to high concentrations [10, 11].

Ginseng has adaptogenic, metabolic, bio-stimulating, anti-emetic and tonic effects, stimulates the appetite. Around the world, ginseng and ginseng roots are widely used to improve mental and physical performance as a tonic and they are an effective tool in the treatment of amnesia [12].

It has been experimentally shown that after oral intake of ginseng powder, a significant improvement is observed in learning and mnemonic processes in old rats of both sexes having a sciatic damage of brain structures [13, 14]. Pharmacological activity is observed due to the content of ginsenosides: saponin glycosides and fatty ester oils, sterols and peptides, vitamins and minerals.

Almost for two thousand years *Rhodiola rosea* (golden root) was used in folk medicine. *Rhodiola rosea* extract optimizes the regenerative processes in the central nervous system, increases efficiency and adaptive capacity of an organism to extreme factors, helps to restore strength after tiredness. The main active ingredient is considered to be glycoside salidroside and its aglycone - tyrosol.

*Rhodiola rosea* roots have tonic, calming, fixing and hemostatic effect.

A long history of taking it as a tonic and an astringent in medicine of Asian countries is presented in fruits of *Schisandra chinensis* being a unique stimulant. This is one of the plants, which is commonly used for the treatment of coronary heart disease. What the magnolia extract activates, are estrogen-dependent luciferase genes from cells transiently transfected with the estrogen receptor [15].

Preparations from *Schizandra* raise excitation in the cerebral cortex and increase the reflex activity of the CNS. Toning, refreshing, stimulating effect of *Schizandra* (Chinese magnolia vine) is especially pronounced during intense mental occupation, what requires concentration, attentiveness, perception of wholeness. It is very important, that its tonic effect is not accompanied by depletion of nerve cells. Ginseng containing beverages increase visual acuity and ability of eyes to adapt to darkness. They reduce the heart rate, increasing its amplitude.

The tonic effect of *Schizandra chinensis* beverages can be used as for practically healthy people (with tiredness, fatigue, reduced performance, lethargy, spring vitamin deficiency), and the people who suffer from hypotension, psychasthenia, vascular dystonia of hypotonic type.

However, despite the widespread consumption of tonic (energy) beverages and numerous studies of various aspects of this problem, the question of the biological safety of prolonged consumption of drinks containing tonic components remain unresolved, for example: of such as caffeine, taurine and herbal extracts - adaptogens.

The purpose of research is to analyze the impact of prolonged use of the main components of tonic

(energy) beverages - caffeine, taurine and herbal extracts (adaptogens in the traditionally recommended amounts) under in vivo conditions in the model object.

### OBJECTS AND METHODS OF STUDY

The study was performed on 150 adult Wistar rats of both sexes (females,  $n = 75$ , male,  $n = 75$ ), weighing  $371 \pm 26$  g (females:  $281 \pm 29$  g; males:  $461 \pm 23$  g) in accordance with the requirements of the content and the humane treatment of experimental animals: Ministry of Health of the USSR from 12.08.1977 № 755 "On measures to further improve the organizational forms of work with the use of experimental animals"; "Rules of work with experimental animals" and "Rules for handling, maintenance, anesthesia and killing of experimental animals", approved by the Ministry of Health of the USSR (1977) and the Ministry of Health of the RSFSR

(1977); principles of the European Convention (Strasbourg, 1986) and the Declaration of Helsinki of the World Medical Association on the humane treatment of animals (1996).

Study design was approved by the local ethics committee of Medical University "Kemerovo State Medical Academy" the Ministry of Health.

Criteria for inclusion animals in an experiment with are as follows: age 3–5 months, weight not less than 250 g and not more than 485 g of active animals without visible traumatic lesions and clinical manifestations of heart disease, liver and kidney.

Exclusion criteria were as follows: age less than 3 and more than 5 months, weighing less than 250 g and 485 g more, less active, painful animals.

All the animals were divided into 5 groups according to consumable components of tonic (energy) beverages (Table 1).

**Table 1.** Characteristics of groups of animals consuming tonic components of (energy) beverages

Group	Components of (energy) beverages	List on sex of animals	
		males	females
Group 1 (control, $n = 30$ )	Distilled boiled water	15	15
Group 2 ( $n = 30$ )	Caffeine and taurine	15	15
Group 3 ( $n = 30$ )	Ginseng herbal extract	15	15
Group 4 ( $n = 30$ )	Rhodiola rosea extract	15	15
Group 5 ( $n = 30$ )	Schizandra chinense extract	15	15

Group 1 (control): animals drank water (previously cleaned by filter "Barrier", heated to boiling and chilled to a room temperature) in amounts corresponding to those in groups 2–5.

Group 2: animals consumed aqueous solution of 0.03% caffeine and 0.25% aqueous solution of taurine (non-alcoholic tonic components of (energy) beverage) "Red Bull" in calculation of 0.04 mg and 0.03 mg per 100 g body weight, respectively.

Caffeine and taurine of brand Esarom (Essenzenfabrik Ges.mbH, Austria) was dissolved in pre-treated (by filter "Barrier"), boiled and cooled to room temperature water.

Group 3: The animals ate aqueous extract of ginseng Panax ginseng (20 g extract 100 ml of water) at the rate of 4 mg of extract per 100 g body weight.

Group 4: animals consumed aqueous extract of Rhodiola rosea, brand Rhodiola rosea (20 g extract 100 ml of water) at the rate of 4 mg of extract per 100 g body weight.

Group 5: animals consumed aqueous extract of Schizandra chinensis (Schizandra chinensis) (20 g extract 100 ml of water) at the rate of 4 mg of extract per 100 g body weight.

The choice of the dose usage in this study was based on recommendations according to the decision of the Customs Union dated 07.04.2011, № 622 and GOST R 52844-2007 "Soft tonic drinks. General specifications".

Animals used the prepared solutions of components tonic (energy) drinks daily in the morning for three weeks.

To put them from the experiment, the animals were given an overdose of diethyl ether (inhalation).

For the investigation samples of mixed blood were collected into sterile tubes containing EDTA. After centrifugation (16 TsLn, Polycom, Russia) at 4500 rev/min. for 10 minutes, plasma was collected into Eppendorf tubes and placed into a freezer, where it was kept at  $-18^{\circ}\text{C}$  before the studies. Only a single freeze-thawing was allowed.

In the blood serum there has been determined alanine aminotransferase aspartamaminotransferase (ALT and AST) activity spectrophotometrically (SF-2000, OKB Spektr, Russia) using reagent kits (JSC "Olvex Diagnosticum", Russia), of creatine kinase (CK) (HUMAN, Germany), of lactate dehydrogenase (LDH), the concentration of urea (JSC "Deacon-DS", Russia), glucose (JSC "Analysis-M", Russia), uric acid ("Vital Development Corporation", Russia), total cholesterol (JSC "Vector-Best" Russia) in accordance with the attached instructions. Thus it proceeded from the fact that more than two-three-fold increase in the activity of any of enzymes identified in blood is sufficiently reliable criterion of cell membranes damage, primarily hepatocytes, cardiomyocytes, as well as skeletal muscles and other cells, to a less extent. Aggregate analysis of changes in the concentrations

of glucose, urea, uric acid in the blood allows to evaluate integrally the metabolic rate, the ratio of anabolic and catabolic processes in the adaptive metabolic reconstructions, and increasing the concentration of total cholesterol - the degree of atherogenic food consumed.

Statistical analysis of the data was performed using the software package Microsoft Office Excel 2003 (license agreement 74017-640-0000106-57177) and Stat Soft Statistica 6.1 (EULA BXXR006 D092218FAN11). The distribution pattern of the data was assessed using the Shapiro-W Wilk. For indicators characterizing the quality of signs, the absolute number and relative value were indicated as a percentage ratio (%). For quantitative characteristics calculating arithmetic meaning (M) and standard deviation the calculation (m) was presented. To test the statistical hypothesis of the equality of the average rank of the two independent samples a Mann-Whitney test (Mann Whitney U-Test) was used.

Comparison of two related groups by quantitative

variables, that are not normally distributed, was performed using Wilcoxon test for paired comparisons (Wilcoxon matched pairs test). In case of normal distribution of the characteristic data they are presented as a mean quantity and standard deviation, with comparisons using student's test for affiliated groups. The critical level of significance is  $p < 0.001$ .

## RESULTS AND DISCUSSION

It was found that after three weeks of daily intake of aqueous solutions of caffeine and taurine, ginseng extract, *Rhodiola rosea* and *Schisandra chinense* extracts at doses corresponding to those in use of tonic (energy) beverages, ALT activity in serum did not change significantly regardless the consumption component and sex of animals (Table 2). However, activity of LDH 2-fold and CK 6-fold on the average increased in the use of aqueous solutions of caffeine and taurine. Activity of AST increased by 25–45% under use of aqueous solutions of caffeine and taurine, extracts of *Rhodiola rosea* and *Schisandra chinense*.

**Table 2.** Effect of prolonged use of restorative components of tonic (energy) beverages on the activity of enzymes, biomarkers of cell damage in blood serum ( $M \pm m$ )

Group	Enzyme Activity U/L			
	ALT	ACT	LDH	CK
1 (control; water)	33.4 ± 0.1	30.8 ± 0.1	268.7 ± 11.5	11.1 ± 0.3
2 (caffeine and taurine)	33.5 ± 0.2	37.2 ± 1.2*	544.2 ± 9.6*	66.6 ± 0.4*
3 (ginseng)	33.1 ± 0.1	30.5 ± 0.2	268.0 ± 11.2	10.6 ± 0.2
4 ( <i>Rhodiola rosea</i> )	35.3 ± 0.3	44.4 ± 0.4*	231.0 ± 6.0*	11.0 ± 0.2
5 ( <i>Schizandra chinense</i> )	37.3 ± 0.2*	43.3 ± 0.1*	249.4 ± 9.6	12.1 ± 0.4

Note. \*  $p < 0.001$  as compared to (group 1).

In most cases, absence of significant pathogenetic (multiple) changes in the activity of investigated enzymes - biomarkers in serum indicated maintaining their intracellular pool and accordingly the functional integrity of cell membranes during prolonged daily use of energy drinks tonic components.

Subclinical increase (average 2-fold) in LDH with usage of aqueous solutions of caffeine and taurine reflected not so much damage of the cell membrane, as the stimulation of physiological and metabolic processes conjugated with the predominance of anaerobic incomplete oxidation of glucose and lactate, increased formation in peripheral tissues and red blood cells, increased gluconeogenesis, including that of lactate. As the consequence of these metabolic rearrangements LDH expression in cells increased, and in these conditions the inevitable increase in its extracellular translocations occurred, the result of which was basically an increase of LDH activity in serum with prolonged use of caffeine and taurine.

However, with a very prolonged usage of aqueous solutions of caffeine and taurine pathogenetically significant (6-fold) increase in CK activity an having adverse effect has been detected, which could not only be the result of physiological rearrangement of metabolism and energy exchange. Moreover, in conjunction with subclinical increase in LDH, it is sufficiently strong reason to believe in the possibility of indirect excessive stimulation of catabolic processes, membrane damage cardiomyocytes, skeletal muscle and possibly to a less extent of hepatocytes during prolonged use of caffeine and taurine in traditionally recommended amounts.

Thus a trend reflecting the increased prevalence of metabolism in catabolic processes with prolonged use of caffeine and taurine was a more pronounced tendency to develop hypoglycemia against the increased formation of urea and uric acid, which reflects the prevalence of protein catabolism and synthesis of their nucleotide compared with the animals, drinking vegetable extracts (Table 3).

**Table 3.** Effect of prolonged use of restorative components of (energy) beverages on integrated indicators of metabolism (M ± m)

Group	Glucose, mmol/l	Urea, mmol/l	Uric acid, mg/100 ml	Total cholesterol, mmol/l
1 (control; water)	4.1 ± 0.1	6.2 ± 0.3	5.9 ± 0.2	1.3 ± 0.01
2 (caffeine and taurine)	3.4 ± 0.01*	9.9 ± 0.2*	7.8 ± 0.3*	1.1 ± 0.01
3 (ginseng)	5.1 ± 0.1*	10.0 ± 0.6*	6.0 ± 0.2	1.2 ± 0.01
4 (Rhodiola rosea)	5.2 ± 0.1*	9.3 ± 0.4*	7.8 ± 0.1*	1.3 ± 0.1
5 (Schizandra chinense)	5.5 ± 0.1*	5.7 ± 0.1	7.5 ± 0.2*	1.5 ± 0.1

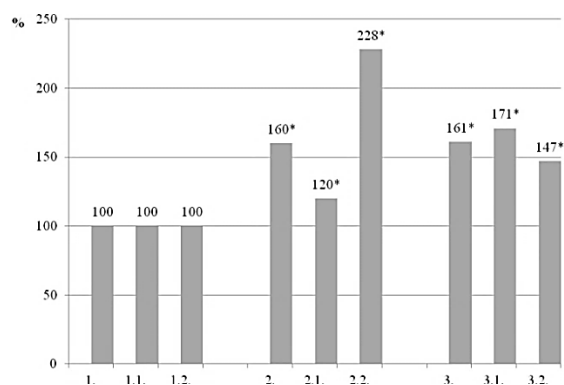
Note. \* p<0.001 as compared to (group 1).

With prolonged daily intake of aqueous solutions of herbal extracts (ginseng, Rhodiola rosea and Schizandra chinensis) metabolic adjustment with the predominance of catabolic processes was carried out as well. However, against the background of increased catabolism of proteins and nucleotides, a reflection of what has been a significant increase in the concentration of urea and uric acid in blood, normoglycemia was maintained. Furthermore, to identify trends in the development of hyperglycemia, especially in the use of the extract of Chinese magnolia vine, with the animals in this group, the urea concentration increase in their blood was observed. Another feature revealed that influence of herbal extracts on the metabolism stated no significant increase in the concentration of uric acid in blood serum with prolonged use of ginseng extract. It may likely be, that at the bottom of the tonic effect of ginseng, Rhodiola and Schizandra there are several different metabolic reconstructions, including the provision of constancy of blood glucose at a higher degree of energy consumption.

Any significant increase in the concentration of total cholesterol in serum with prolonged use of aqueous solutions of caffeine with taurine, ginseng extract, Rhodiola and Schizandra have been identified, suggesting that the lack of direct atherogenic effect in all investigated in this study tonic components of (energy) beverages.

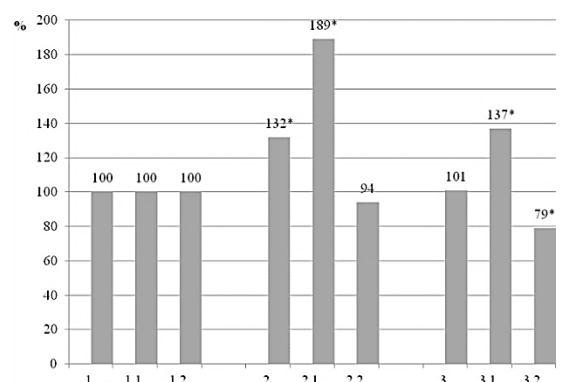
Taking into account the literature on gender peculiarities of tonic (energy) beverage consumption, an attempt was made for experimental evaluation of the influence of features of the major components on biomarkers of cell membrane integrity and integral indicators of metabolism depending on the sex of the animals.

Comparative analysis of the data obtained allowed to identify very important gender-specific peculiarities. In particular, when using an aqueous solution of caffeine with taurine greater concentrations of urea increased significantly in females, and in males - during prolonged use of an aqueous solution with ginseng extract (Figure 1). With prolonged use of aqueous solutions of caffeine with taurine and ginseng extract concentration of uric acid by males was significantly increased (Figure 2), whereas changes in the activity of enzymes, biomarkers of cell membrane integrity and some other features of males did not differ significantly, as well as the concentration of glucose.



Note. \* p < 0.001 compared with control group (Group 1).

**Fig. 1.** The effect of prolonged use of restorative components of (energy) beverages on the concentration of urea in the blood serum (% of control): (1.) Group 1 (control; water); (1.1.) Group of control. Males water intake; (1.2.) Group of control. Females water intake; (2.) Group 2; (2.1.) Females water solution intake with caffeine and taurine; (2.2.) Females water solution intake with caffeine and taurine; (3.) Group 3; (3.1.) Males water solution intake of ginseng extract; (3.2.) Males water solution intake of ginseng extract.



Note. \* p < 0.001 compared with control group (Group 1).

**Fig. 2.** The effect of prolonged use of restorative components of tonic (energy) beverages on the concentration of uric acid in the blood serum (% of control): (1.) Group 1 (control; water); (1.1.) Group of control. Males water intake; (1.2.) Group of control. Females water intake; (2.) Group 2; (2.1.) Females water solution intake with caffeine and taurine; (2.2.) Females water solution intake with caffeine and taurine; (3.) Group 3; (3.1.) Males water solution intake of ginseng extract; (3.2.) Males water solution intake of ginseng extract.

The concentration of urea in the blood serum of males with prolonged use of aqueous solutions in combination with caffeine and taurine as well as ginseng extract increased after 3 weeks 1.2 and 1.7 times on average respectively, as compared with the control group. With females, who consumed caffeine with the aqueous solution of taurine, this concentration increased twice more. After the use of ginseng it increased 1.5 times more. This could be due to gender characteristics of the hormonal status - prevalence of catecholamine stimulation and more muscle mass inherent to males.

However, the concentration of uric acid in the prolonged use of aqueous solutions of caffeine in combination with taurine increased significantly greater in males, possibly due to the increased exogenous methylated xanthine biotransformation in the liver.

With prolonged use of aqueous extract of ginseng gender differences in changes of uric acid concentration as well as the urea concentration in blood serum were although less pronounced, but nevertheless, concentration of urea increased in males more.

Thus, the comparative analysis of the results together with literature data allows us to conclude that, due to significantly higher body weight of males ( $461 \pm 23$  g and  $281 \pm 29$  g, male and female respectively) and increased sympathetic-adrenal activity, stimulating effect of caffeine is accompanied by a pronounced intensification of metabolism and energy consumption, which inevitably leads to an increased consumption of glucose. To ensure constancy of glucose in these conditions, additional sources of initial substrates of gluconeogenesis from protein preparation are necessary, the formation of which from proteins and nucleotides is accompanied by the formation of final products of catabolism - urea and uric acid, a more

pronounced increase in the concentration of the latter in males may result from rapid caffeine metabolism in a liver. The authors do not exclude, that due to it the fact of a more pronounced stimulatory effect of caffeine-containing soft beverages in men compared to women [4] is stipulated.

## CONCLUSION

A prolonged (21 day) daily intake of aqueous solutions of extracts of ginseng, *Rhodiola rosea* and Chinese Schizandra in amounts corresponding to those in the use of tonic (energy) beverages, proved not to be accompanied by significant changes in pathogenesis of integrated metabolic parameters and biomarkers of cell membrane integrity, that is biologically safe.

(1) Prolonged daily use of aqueous solutions of caffeine with taurine in amounts, corresponding to those in the use of tonic (energy) beverages, carries a risk of structural and functional disorders of organs and tissues due to damage of the cell membrane mediated by excessive stimulation of energy metabolism and metabolism with a predominance of catabolic processes.

(2) Changes in metabolic rate with prolonged daily use of aqueous solutions of caffeine with taurine and ginseng extract are gender-sensitive, due to differences in body weight, respectively, the level of energy metabolism, gluconeogenesis and ensure its initial substrate due to enhanced catabolism of proteins and nucleotides, more pronounced in males.

(3) Long-term use of aqueous solutions of caffeine with taurine requires clinical and laboratory monitoring due to the risk of structural and functional disorders of organs and tissues as a result of the predominance of catabolic processes.

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