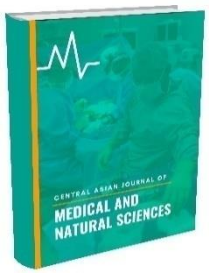
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 06 | Nov-Dec 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Antihypoxic Activity of Tribulus Terrestris L. Extract in Experimental Animals

1. **Abdinazarov Ibrokhim Tuychievich**
2. **Sanoev Zafar Isomiddinovich**
3. **Khamroev Tolmas Tolibovich**
4. **Khazhibaev Timur Atakhanovich**
5. **Rashidov Sohib Zamon o‘g‘li**
6. **Rakhimboev Sukhrob Davlatyor o‘g‘li**

Received 2nd Oct 2023,
Accepted 19th Nov 2023,
Online 29th Dec 2023

[1,2,3,4,5,6] Institute of Chemistry of Plant Substances named after Academician S.Yu. Yunusov of the Academy of Sciences of the Republic of Uzbekistan

Abstract: Effective doses were selected after studying the activity of a dry extract of the Tribulus terrestris L. plant growing in the territory of the Republic of Uzbekistan, based on screening studies on experimental animals. Its antihypoxic activity was evaluated using models of normobaric, heme, and cytotoxic hypoxia. According to the results obtained, the dry extract of the Tribulus terrestris L. plant is not inferior in antihypoxic activity when taken orally at doses of 10, 30 and 60 mg/kg to comparable drugs L-carnitine and mildronate, justified in experiments.

Key words: dry extract of Tribulus terrestris L. plant, hypoxic, hemic, cytotoxic, sodium nitrite, sodium nitroprusside.

Relevance. The development of effective methods to increase the body's resistance to hypoxia is an urgent task of modern medicine. The term "hypoxia" refers to the absolute or relative insufficiency of energy supply for the optimal functioning of organs and tissues of the body and the intensity of plastic processes in them. This condition leads to a violation of the vital functions of the body as a whole [1]. Hypoxia is a pathological condition that occurs as a result of insufficient oxygen supply to tissues or impaired cell destruction and occurs in significant conditions caused by various external and internal factors, including disease or damage to organs and tissues. The issues of hypoxia and antihypoxants are well covered in the fundamental works of a number of scientists [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Oxygen is necessary for oxidative phosphorylation reactions in the respiratory chain and oxygenase reactions in the mitochondrial electron transfer system, for the formation of free radical oxidation, for the formation of reactive oxygen species and other processes. There are several main types of hypoxia: exogenous (hypoxic), respiratory (respiratory), circulatory (cardiovascular), hemic, tissue and mixed. They also describe endogenous hypoxia caused by pathological processes and diseases, and distinguish between rapid (seconds, minutes), acute (hours) and chronic hypoxia (for example, heart failure, atherosclerosis of the brain, dyscirculatory encephalopathy) depending on the time of its

development [13]. Hypoxia is a pathological process characterized by a lack of biological oxidation, leading to a violation of the energy supply of functions and plastic processes in the body. Hypoxia is a condition that causes serious problems for the human body, causing the appearance and/or development of neurological diseases. Hypoxia in the aging process is especially important for age-related neurodegenerative diseases [14]. Aging is characterized by progressive functional decline [15], which can be changed by changing the availability of O_2. Despite the important role of O_2 in oxidative metabolism, a moderate decrease in O_2 concentration may benefit aging organisms. The antihypoxic property was found in anthracene derivatives, saponins, coumarins, flavonoids, phenolic alcohols and their glycosides and other biologically active substances contained in medicinal plants [16]. In particular, it was found that the *Tribulus terrestris L*. plant growing on the territory of Uzbekistan practically does not differ in chemical composition and properties from a foreign plant species, and technological processes have been studied [17], and studies of its various pharmacological activity are currently underway. In this article, the antihypoxic activity of *Tribulus terrestris L.* plant extract in experimental animals was studied.

Materials and methods of research. The experiments were conducted on white mongrel male mice weighing 20-22 g, kept in standard vivarium conditions for free access to water and food. All animal experiments were conducted in accordance with the requirements of the international recommendations of the European Convention for the Protection of Vertebrates [18]. *Tribulus terrestris* furostanol saponins can be isolated from ballast substances by liquid-liquid extraction, which changes the polarity of the extractor depending on the dynamics of the process. Chloroform treatment allowed the removal of the maximum amount of lipid-like compounds and showed minimal losses of furostanolic saponins, and subsequent treatment with ethyl acetate restored low-polar substances. To extract furostanolic saponins from an aqueous solution, four-fold extraction with butanol was used. Optimal conditions for aerosol drying of furostanolic saponins have been developed. The highest yield of ”dry tribulus extract" was obtained at a solution feed rate of 80 l/h, a spray head rotation speed of 8000 rpm and a coolant speed of 2000 kg/h. A technology for the production of “Tribulus dry extract” containing at least 45% of furostanolic saponins has been developed from *Tribulus terrestris*. Experiments were conducted on the basis of this dry extract. The antihypoxic activity of the extract was evaluated in accordance with the ”recommendations on preclinical drug activity" [19]. Acute normobaric hypoxia with hypercapnia was carried out in experiments on white male mice with the same body weight (body weight difference of no more than 2 g per group) by placing 2 experimental animals in hermetically sealed jars with a volume of 250 cm3. Cytotoxic hypoxia was caused by subcutaneous injection of sodium nitroprusside at a dose of 20 mg/kg body weight once in mice, while hemic hypoxia was caused by injection of sodium nitrite at a dose of 300 mg/kg into the abdominal cavity. The criteria for evaluating the antihypoxic effect of the studied substances on all models of hypoxia were evaluated depending on the life expectancy of the experimental animals. The studied substances were administered orally 60 minutes before the experiment and the use of hypoxants. The effectiveness of the studied drugs was evaluated in comparison with mildronate and L-carnitine. The obtained results were processed by statistical analysis methods [20].

The results obtained. We can see that the antihypoxic activity of *Tribulus terrestris* extract showed higher activity compared to control and comparable drugs when studies were conducted on white mice on different models of hypoxia at different doses.

1. We know that in the pathogenesis of normobaric hypoxia, there is a decrease in oxygen flow to organs due to arterial hypoxemia, hypocapnia, gaseous alkalosis and increased oxygen demand caused by arterial hypotension. Initially, the examination was performed on a model of normobaric hypoxia. The results obtained are shown in Table 1.

Table 1. Investigation of the antihypoxic properties of *Tribulus terrestris* extract on a model of normobaric hypoxia

Substance	Dose in mg/kg	Average life expectancy (per minute)	Effect against hypoxia (in %)
Control (dis.wat)	0.2 ml	26.5±2.8	-
Tribulus terrestris extract	10	36.5±3.1*	37.73
	30	38.0±3.4*	43.39
	60	43.5±4.2*	64.15

Note: *P≥0.05 compared to the control group

According to the results obtained, we can see that *Tribulus terrestris* extract increased life expectancy by 10; 37.73%; 43.39% and 64.15%, respectively, compared with the control group at doses of 60 mg/kg. In this model, we see that in white mice, *Tribulus terrestris* extract causes an increase in life expectancy by reducing the possible pathogenesis conditions of the above normobaric hypoxia.

2. Hemic hypoxia is considered a type of endogenous hypoxia and occurs with a decrease in blood oxygen capacity due to a decrease in the amount of hemoglobin (HB) per unit volume of blood and a violation of the transport properties of HB. The hemic type of hypoxia is characterized by a decrease in the ability of HB in erythrocytes to bind oxygen (in the capillaries of the lungs), transport and release its optimal amount into tissues. A decrease in hemoglobin content per unit volume of blood and in the body as a whole is observed with a significant decrease in the number of red blood cells and/or a decrease in hemoglobin content (sometimes up to 40-60 g / l), i.e. it can manifest severe anemia. Our further research was based on the study of the activity of *Tribulus terrestris* extract in various doses on a model of heme hypoxia. The experiments used doses of 10, 30 and 60 mg/kg of *Tribulus terrestris* extract, as well as doses of 50 and 100 mg/kg of mildronate and 150 mg / kg of L-carnitine with high activity of comparable drugs; Comparative studies of doses of 300 and 500 mg/kg were conducted. The results obtained are shown in Table 2.

Table 2. Investigation of the effect of *Tribulus terrestris* extract on animal life expectancy on a model of hemic hypoxia

Substance	Dose in mg/kg	Average life expectancy (per minute)	Effect against hypoxia (in %)
Control (sodium nitrite)	300	14.25±1.3	-
Tribulus terrestris extract	10	21.0±2.1*	47.36
	30	22.75±2.1*	54.23
	60	23.5±2.4*	64.91
Mildronate	50	15.75±1.7*	10.5
	100	18.5±1.6*	29.82
L-carnitine	150	15.82±1.7*	11
	300	16.47±1.9*	15.6
	500	15.59±1.5*	9.4

Note: *P≤0.05 compared to the control group

According to the results, *Tribulus terrestris* extract increased the life expectancy of white mice by 10; 47.36%; 54.23% and 64.91%, respectively, at doses of 30 and 60 mg/kg, and also showed higher activity than these comparable drugs. The above doses were given for 10 days, and then studied using

sodium nitrite, causing hemic hypoxia, given that these experiments showed high results with a single administration of the compound under study.

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Table 3. Investigation of the effect of *Tribulus terrestris* extract on animal life expectancy on a model of hemic hypoxia when taken for 10 days

Substance	Average life expectancy (per minute)	Effect against hypoxia (in %)
Control (sodium nitrite 300 mg/kg)	18.4±1.8	-
Tribulus 10 mg/kg	17.8±1.4*	-3.26
Tribulus 30 mg/kg	19.8±1.9*	7.608
Tribulus 60 mg/kg	19.2±1.7*	4.35

Note: *P≤0.05 compared to the control group

From the result obtained in Table 3, it can be seen that the continued administration of the extract does not bring sufficient benefit, given that in this model the extract showed almost the same activity as the control group.

3. Cytotoxic hypoxia occurs due to the inability of these tissues to use oxygen supplied through the blood. In experiments, *Tribulus terrestris* extract was orally administered at doses of 10, 30 and 60 mg/kg, evaluating the effect on life expectancy in white mice on a model of cytotoxic hypoxia. The results are presented in table 4.

Table 4. Investigation of the effect of *Tribulus terrestris* extract on animal life expectancy on a model of cytotoxic hypoxia

Substance	Dose in mg/kg	Average life expectancy (per minute)	Effect against hypoxia (in %)
Control (Sodium Nitroprusside)	20	22±2.1	-
Tribulus terrestris extract	10	30.4±2.9*	38.18
	30	30.4±2.4*	38.18
	60	33.2±3.1*	50.9
L-carnitine	150	22.8±2.7*	3.63
	300	24.0±3.4*	9.10
	500	21.8±2.8*	-0.90

Note: *P≤0.05 compared to the control group

Based on the results obtained, we can see that in the cytotoxic hypoxia model, *Tribulus terrestris* extract showed higher activity compared to the control group and the comparable drug L-carnitine in all doses.

Discussion. The state of hypoxia that occurs in the body plays an important role in accelerating the aging process of cells, as well as in the occurrence of various diseases. Conditioning in hypoxia offers a promising strategy for the treatment of diseases of the central nervous system [21]. Data on the improvement of cognitive functions in elderly people with hypoxia [22-26] confirm the use of periodic condensed hypoxia for the treatment of neurodegenerative diseases. Hypoxic exercises have already proven to be beneficial in terms of mood, fitness, and metabolism in patients with multiple sclerosis [27]. Based on the above literature data, it can be seen that hypoxia plays a key role in the development of not only aging processes, but also neurodegenerative diseases. In the experiments conducted, *Tribulus terrestris* extract showed that in all models of hypoxia, comparable drugs are not

inferior to mildronate and L-carnitine. Given these circumstances, in the future it will be possible to use *Tribulus terrestris* extract as an antihypoxic agent in order to prevent and reduce the aging process, as well as the development of various neurodegenerative diseases.

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