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Toxicological Characteristics Of N-Deacetyllappaconitine Under Chronic Administration In White Rats

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ABSTRACT

In chronic experiments in laboratory animals, studied toxicological perspective new antiarrhythmic effect of N-deacetyllappaconitine for the treatment of arrhythmic states, an original herbal preparation based on Aconitum leucostonum, Ac.Septentrionale was created. During 2,5 months of intragastric administration in animals exposed to all doses of N-deacetyllappaconitine, no deviations in the parameters of the functioning of the nervous system were found, therefore, this value is the lowest effective (threshold) dose in a chronic experiment.

KEYWORDS

N-deacetyllappaconitine, chronic experiments, central nervous system, biochemical parameters

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INTRODUCTION

Heart rhythm disorders are one of the most complex and urgent problems of modern cardiology. Arrhythmias are detected both in various cardiovascular diseases [1-4] and in healthy individuals [5, 6]. The spectrum of clinical manifestations of cardiac arrhythmias varies from asymptomatic to severe [7, 8], from prognostic insignificant arrhythmias to determining the nature of a long-term outcome [9-12]. As a result of the development of new antiarrhythmic agents N-deacetyllappaconitine [13, 14] for the treatment of arrhythmic conditions, the scientists of the Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan created an original herbal preparation based on Aconitum leucostonum, Ac.Septentrionale. In addition, you need to remember that antiarrhythmic drugs often show side effects. Many antiarrhythmic agents are more or less characterized by an arrhythmogenic effect, can cause disorders of the nervous system, changes in the blood in the form of hematological and biochemical indicators. A special role in conducting such studies belongs to the study of the chronic toxicity of the compound in warm-blooded animals, as a result of which the values of threshold and subthreshold doses are established.

The aim of the work was to determine the toxic and low-acting doses of N-deacetyllappaconitine in a chronic experiment on white rats.

MATERIALS AND METHODS

The experiments were performed on 24 random-bred white male rats with an initial weight of 170-220g. Healthy animals with a clean coat after intragroup adaptation were selected for the experiment. The treatment of animals was consistent with the ethical principles of good laboratory practice [15]. N-deacetyllappaconitine was injected into the stomach of experimental animals using a needle probe. Distilled water was used as the solvent.

The study of toxic properties was carried out by administration of fixed doses of N-deacetyllappaconitine to male white rats for 2.5 months. The animals were divided into 4 groups of 6 individuals each: I-control and II, III, IV-experimental, exposed to 1/1100, 1/11 and 1/4. 4 of LD50 (0.1; 10 and 25 mg/kg, respectively). The control group was injected with distilled water in equivalent volumes.

During the experiment, changes in the body weight of the animals were recorded. During and after the experiment, a number of indicators of behavioral activity were studied in rats using the previously studied method [16] in the "Open Field" setup. After simultaneous decapitation of rats during autopsy, the relative mass coefficients (OCM) of internal organs – heart, kidneys, liver, spleen, thymus, adrenal glands, lungs-were determined. To characterize the functional state of the body of experimental animals, the morphofunctional composition of peripheral blood was studied (hematological analyzer Mythic18, Switzerland), and a number of biochemical parameters of blood serum were determined (automatic biochemical analyzer Accent 200, Poland).

The results of the studies were processed using the generally accepted methods of variation

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statistics. The Student's parametric t-test with the Bonferroni correction or the nonparametric Mann-Whitney U-test were used to assess the differences between the groups. The critical level of significance when testing statistical hypotheses was assumed to be $p \leq 0.05$.

RESULTS

During the experiment, the general condition and behavior of the animals were monitored, and body weight was determined weekly. After the end of the experiment period, the animals were decapitated, hematological, biochemical and pathomorphological studies were performed.

The results of the conducted studies showed that all experimental rats in doses of 0.1-10 mg / kg inside well tolerate the drug in these doses. The general condition and behavior of the experimental animals did not differ significantly from the control rats. A slight decrease in the body weight of rats in the first weeks was observed in both the experimental and control groups. In the remaining periods of administration, all experimental and control animals ate food well, gained weight. In groups of 25 mg/kg closer to toxic doses, 1 out of 6 animal deaths were observed.

Chronic administration of N-deacetyllappaconitine at doses of 25, 10 and 0.1 mg / kg did not cause significant changes in the condition of the experimental animals, and during the experiment, the experimental animals did not differ from the control animals in terms of body weight gain. The functional state of the central nervous system of experimental animals in a chronic experiment was evaluated by the parameters of their behavioral activity. When N-deacetyllappaconitine was administered at all doses, statistically significant changes in the behavior of animals of the experimental groups were noted, expressed according to the studied behavioral indicators in a significant increase from control rats both on day 30 of administration (table 1) and repeated on day 60 of administration (Table 2).

Table 1

Indicators of behavior of white rats when administered N-deacetyllappaconitine in a chronic experiment 30 day administration n=6

The comparison group	Indicators					
	pass through sectors	mink reflex	plumbing	grooming	stool	urine
I- control	12,3±2,58	12,17±4,3	1±0,43	0,5±0,43	2,17±2,15	0,6±0,4
II - 0,1 mg/kg	22,4±3,87*	23,8±4,3*	2,1±0,72*	0,38±0,4	2,8±1,7	0,1±0,4*
III - 10 mg/kg	17,7±6,88*	14,3±5,16	1,4±2,58	0,72±0,43	3,8±2,15	0*
IV - 25 mg/kg	13,7±4,73	11,5±3,8	1±0,43	0,45±0,4	2,1±1,3	0*

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Note. * - differences are statistically significant, p≤0.05

Table 2

Indicators of behavior of white rats when administered N-deacetyllappaconitine in a chronic experiment day 60 administration n=6

The comparison group	Indicators					
	pass through sectors	mink reflex	plumbing	grooming	stool	urine
I- control	9,2±2,15	10,8±3,44	0,4±0,43	0,17±0,43	1,3±0,43	2,8±1,3
II - 0,1 mg/kg	21,5±5,16*	22,1±7,3*	2,1±0,86*	1±0,43*	0,33±0,43*	0,3±0,4*
III - 10 mg/kg	17,1±3,44*	23±2,58*	1,3±0,43*	1,17±0,43*	0,67±0,43	0,83±0,86*
IV - 25 mg/kg	14,3±4,3*	18,3±3,1*	0,7±0,43*	0,67±0,4*	0,5±0,43*	1±0,43*

Note. * - differences are statistically significant, p≤0.05

Administration of N-deacetyllappaconitine at doses of 0.1, 10, and 25 mg/kg for 70 days did not cause changes in the OCM of internal organs – heart, liver, kidneys, lungs, spleen, and adrenal glands in rats compared to control animals. Macroscopic examination of the internal organs also showed that the drug does not cause general pathological and specific destructive changes in the organs and tissues of animals, the condition of the internal organs in the experimental and control rats corresponded to the norm (Table 3).

Table 3

Relative mass coefficients of internal organs of rats with the introduction of N-deacetyllappaconitine in a chronic experiment g / kg

RMC bodies	I-control	II-experimental 0.1 mg / kg	III-experimental 10 mg / kg	IV-experimental 25 mg / kg
Liver	6,9±0,88	7,12±0,6	7,15±1,0	7,3±1,1
Renals	1,47±0,13	1,52±0,2	1,58±0,13	1,6±0,2
Suprarenal	0,058±0,02	0,06±0,008	0,061±0,01	0,06±0,01
Spleen	0,91±0,1	0,9±0,17	1,05±0,21	1±0,36
Heart	0,71±0,08	0,87±0,13	0,91±0,08	1,0±0,16
Light	2,27±0,3	2,2±0,69	2,5±0,3	2,5±0,5
Thymus	0,17±0,06	0,16±0,07	0,16±0,03	0,18±0,05

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Subsequently, a study of blood cells was carried out, which showed that N-deacetyllappaconitine at doses of 0.1, 10 and 25 mg/kg does not have a negative effect on the functioning of the "red sprout"system. Similarly, in all experimental groups, the values of platelet parameters corresponded to the control values. Also, no statistically significant changes in the leukogram were found in comparison with the control group of animals (Table 4). The results of the conducted biochemical studies are presented in Table 5.

Table 4

Effect of N-deacetyllappaconitine on rat hematological parameters on the 1st, 30th, and 70th day of the study

Days	Indicators	Control	0,1 mg/kg	10,0 mg/kg	25 mg/kg
1-day	Hemoglobin g/%	12,52±1,3	12,43±0,8	12,1±1,3	12,2±0,6
	Red blood cells million/mm3	7,16±0,8	7,05± 1,3	7,25±1,6	7,05±0,8
	White blood cells thousand/mm3	12,52±1,2	12,5±1,7	12,8±1,3	12,9±0,7
30-day	Hemoglobin g/%	12,8±1,3	12,68±1,9	13,88±1,8	13,5±2,3
	Red blood cells million/mm3	7,16±0,9	7,15±0,1	7,18±0,43	7,16±0,43
	White blood cells thousand/mm3	12,52±0,6	12,5±2,1	13,16±0,8	13,6±0,8
70-day	Hemoglobin g/%	12,32±1,6	11,73±0,43	12,3±2,3	13,5±0,43
	Red blood cells million/mm3	7,0±1,43	7,25±0,3	6,86±0,3	7,05±0,1
	White blood cells thousand/mm3	13,0±1,8	12,44±2,3	12,52±1,2	12,67±2,2

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The study of peripheral blood (blood triad: hemoglobin, white blood cells, red blood cells) did not reveal significant differences between the animals of the experimental groups and the control ones at the 70-day administration of the drug. The content of white blood cells tended to decrease, which may be associated with some functional suppression of the bone marrow accompanied by a violation of blood distribution or increased destruction of white blood cells to remove them from the body.

Table 5

The effect of the drug N-deacetyllappaconitine on the biochemical parameters of rats in the blood and organs on the 70th day of the study

Indicators	Control	0,1 mg/kg	10,0 mg/kg	25 mg/kg
Total protein (g / l)	68,2 ±0,2	69±0,1	65,6±0,2	62,7±0,3
AST E/l	45±2,1	46,1±1,8	46,6±2,1	42,8±3,2
ALT E/l	62±1,6	67±2,3	65,5±3,1	58,7±3
Glucose (mmol / L)	4,2±0,2	4,3±0,1	4,4±0,2	4,15±0,3
Cholesterol mg / dl	112±7,8	123±12,1	105,2±4,3	92,7±5,8
Glycogen Liver mg/%	1835,6±43,7	2645,4±28,5	3483,3±48,7	2227,2±38,4
Glycogen Hearts of mg/%	122,9±8,7	134,4±12,4	112,4±9,7	104,3±7,6

DISCUSSION

The analysis of biochemical parameters in animals treated with N-deacetyllappaconitine for 70 days did not reveal significant changes in the activity of ALT and AST, cholesterol, in the blood serum, which indicates the absence of functional disorders of metabolic processes in the tissues.

After hematological and biochemical studies, all groups of rats were decapitated, and macroscopic changes in vital organs were evaluated. We received the parts of important organs, put them in formalin solutions and sent them to the histologist for further histological examination.

CONCLUSION

Thus, the drug N-deacetylappaconitine at 70 days of administration to rats at doses of 0.1 and 10 mg / kg does not cause significant changes in the clinical and biochemical parameters in the body.

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