UDC 615.21: 615.076.9

APPROVED
General Director
CJSC “Saint Petersburg Institute of Pharmacy”
Doctor of Medical Sciences, Professor
[Signature] V.G. Makarov
24.11.2021

It is carried out in accordance with the GLP OECD Principles

STUDY REPORT

Study of the toxic properties of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats

(final)

Number and date of the agreement	**№ 0109-SPIF/2021 dated June 01, 2021**
Study code (BEC)	3.28/21
Study sponsor	Normopharm LLC 121205, Moscow, Territory of the Skolkovo Innovation Center, Nobel Street, 5, floor 1, office 40, room 3 Tel.:+7(915) 465-21-69
Research institution	CJSC “Saint Petersburg Institute of Pharmacy” 188663, Russia, Leningrad Region, Vsevolozhsky District, Kuzmolovsky, Zavodskaya Str., 3, bld. 245. Tel.: +7(812) 603-74-28
Date of the final report	November 08, 2021

Study Leader ***[Signature]*** ***A.V. Popova***

Leningrad Region
2021

<table>
<tr><td>Study title</td><td colspan="6">Study of the toxic properties of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats</td></tr>
<tr><td>Study code</td><td colspan="6">3.28/21</td></tr>
<tr><td>Study goal</td><td colspan="6">Study of the toxic properties of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats</td></tr>
<tr><td>Study objectives</td><td colspan="6">1. study of toxic properties of the test object with analysis of the clinical picture of intoxication;
2. evaluation of the local irritant action;
3. determination of the mean lethal dose (LD_{50}) of the test object.</td></tr>
<tr><td>GLP compliance</td><td colspan="6">Yes, except for the fact that no identity, purity, and stability studies of the test object were carried out by the research institution. These values were determined by the Sponsor according to standard methods.</td></tr>
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<tr><td>Control substance (Vehicle) [M-3.28/21]</td><td colspan="6">Saline solution (Gematek LLC, Russia)</td></tr>
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<tr><td>Route of administration of the test object planned in clinical practice</td><td colspan="6">Per os</td></tr>
<tr><td>Test system, number</td><td colspan="6">Wistar rats (40 males and 40 females)</td></tr>
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<tr><td rowspan="2">1</td><td rowspan="2">Saline solution M-3.28/21</td><td rowspan="16">Intravenous, single</td><td>males</td><td>5</td><td rowspan="2">0</td></tr>
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<tr><td rowspan="2">2</td><td rowspan="14">Lithium Ascorbate T-3.28/21</td><td>males</td><td>5</td><td rowspan="2">3000 a</td></tr>
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<tr><td rowspan="2">8</td><td>males</td><td>5</td><td>3250 c</td></tr>
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</table>

	Remarks 1-$_a$ - doses are given on the basis of Amendment № 1 dated 16.08.2021. Doses of 1000 mg/kg and 2000 mg/kg did not cause lethal effects.

	Based on these data, the study dose range was changed to determine LD_{50}: instead of the planned doses of 125 mg/kg and 250 mg/kg, male and female rats were administered with doses of 3000 mg/kg and 4000 mg/kg, respectively. 2 - [b] - the dose is given on the basis of Amendment № 2 of 15.09.2021. A dose of 3000 mg/kg did not cause lethal effects, and a dose of 4000 mg/kg resulted in the death of 100% of animals. In this regard, based on Supplementary Agreement № 1 and Amendment № 2, 2 additional groups of animals were introduced into the experiment. 3 - [c] - doses are given on the basis of Amendment № 3 of 27.09.2021. A dose of 3500 mg/kg led to the death of 3 males, mortality in females was not observed. Based on these data, Amendment № 2 was issued, according to which the dose for males of group № 8 was 3250 mg/kg, and the dose for females of group № 8 was 3750 mg/kg.

Manipulations	Manipulation	Experiment days
	Body weight recording	Days 1, 2, 8, 15
	Administration of the test object or control substance	Day 1
	Clinical examination	Pre-dosing, days 2, 8, 14
	Clinical observation	Pre-dosing, days 1-15
	Euthanasia of all animals	Day 15
Range of body weight values at the beginning of the experiment:	males - 196.6±2.06 g, females - 176.6±1.40 g	
Route of administration of the test object in the experiment	Intravenous	
Frequency of administration	Once, if necessary, fractionally	
Dosing volume	Dose of the test object	Volume of administration of the substance in a concentration of 125 mg/ml
	500 mg/kg	4 ml/kg
	1000 mg/kg	8 ml/kg
	2000 mg/kg	16 ml/kg
	3000 mg/kg	24 ml/kg (12 ml in fractions)
	3250 mg/kg	26 ml/kg (13 ml in fractions)
	3500 mg/kg	28 ml/kg (divided into 14 ml)
	3750 mg/kg	30 ml/kg (15 ml in fractions)
	4000 mg/kg	32 ml/kg (16 ml in fractions)
Duration of the experiment	15 days	
Key study results		
Lethality	*Intravenous administration:* Dose 500 mg/kg - not recorded	

	Dose 1000 mg/kg - not recorded Dose 2000 mg/kg - not recorded Dose 3000 mg/kg - not recorded Dose 3250 mg/kg - 3 males Dose 3500 mg/kg - 3 males Dose 3750 mg/kg - 5 females Dose 4000 mg/kg - 5 males and 5 females Mortality was predominantly delayed (3-5 days after administration)
LD_{50}	Males - 3225±95.2 mg/kg Females - 3625±62.5 mg/kg
Clinical picture of intoxication	Doses of 0 mg/kg, 500 mg/kg - no lethal outcomes and signs of intoxication were recorded throughout the experiment. Dose 1000 mg/kg - within the first hour after administration, ruffled hair was observed in the animals, inhibition of the general condition was observed in one female. In most animals, signs of intoxication were completely resolved within 2-3 hours after administration. Dose 2000 mg/kg - within the first hour after administration, ruffled hair was observed in the animals, inhibition of the general condition was observed in most females. Signs of intoxication were completely resolved within 2 hours of administration. Dose 3000 mg/kg - during the first hour, all animals had ruffled hair and inhibition of behavior. Then the condition of the animals gradually returned to normal. The ruffled hair was observed until the 7th day of the experiment, then the condition of all animals completely normalized. Dose 3250 mg/kg (males) – most intensive signs of intoxication in males were observed on the 2nd day of observation. Signs of intoxication: ruffled hair, inhibition of the general condition, in isolated cases shortness of breath, discharge from the nose and eyes, tremor. In the survived animals, the condition completely returned to normal on the 10th day of the experiment. Dose of 3500 mg/kg - maximum intoxication - on the 3rd day of observation. Signs of intoxication: ruffled hair, inhibition, in isolated cases discharge from the nose and eyes. In survived females, signs of intoxication persisted until the 7th day of observation, in males, signs of intoxication were observed until the 15th day. Dose 3750 mg/kg (females) – most intensive signs of intoxication in animals were observed on the 3rd day of observation. Intoxication manifested itself in inhibition of various degrees of severity, ruffled hair, discharge from the nose and eyes. In isolated cases, ataxia and tremor were observed. By the 6th day of observation, the mortality rate was 100% Dose 4000 mg/kg - after administration, inhibition of the general condition of various degrees of severity, ruffled hair, ptosis, increased reaction to stimuli (females) were noted in animals. Signs of intoxication were increasing up to the death of animals. By the 5th day, the mortality rate reached 100%.

Effect of the test object on body weight	Control group, doses 500 mg/kg, 1000 mg/kg, 2000 mg/kg - positive dynamics of body weight during the experiment. Dose 3000 mg/kg, males and females - weight loss on day 2 of the experiment compared to the baseline values, then (days 8 and 15) the restoration of the positive dynamics of body weight. Dose 3250 mg/kg, males – the decrease in body weight on the day 2 of the experiment compared to the baseline values, in survived animals - restoration of positive dynamics of body weight (8, 15 days). Dose 3500 mg/kg, males, females - weight loss on the 2nd the day of the experiment compared to the baseline values, then (the 8th and 15th days) the restoration of the positive dynamics of body weight. Dose 3750 mg/kg, females showed a decrease in body weight on the 2nd day of the experiment compared to the baseline values. Dose 4000 mg/kg, females - decrease in body weight on day 2 of the experiment compared to the control group, decrease in body weight on day 2 compared to baseline values. Dose 4000 mg/kg, males - weight loss on day 2 compared to baseline values.
Pathomorphological examination	Doses 500, 2000, 3000, 3250, 3750 mg/kg, routine necropsia: No clinically significant pathological changes in the internal organs were found. Dose 1000 mg/kg, routine necropsia: - one female had plethora of the spleen. Dose 3250 mg/kg, dead animals - in all dead animals, cerebral edema and plethora of its membranes, pulmonary edema and congestion of internal organs were found. In two males, hemorrhages in the stomach, small and large intestines, necrosis of the epithelium of the intestinal mucosa were found. One male has a stomach ulcer. In a single case, the male was found to have hepatic congestion and diffuse small-droplet fatty dystrophy. The cause of death of all dead animals in this group was acute heart failure. Dose 3500 mg/kg, dead animals - in all dead animals, cerebral edema and plethora of its membranes, pulmonary edema and congestion of internal organs were found. Two males also had chronic lobular purulent bronchopneumonia, and one male had extensive hemorrhages and focal pleurisy and adhesions of the caudal lobes of the lungs to the dorsal part of the pleura. The cause of death of all dead animals in this group was acute heart failure. Dose 3750 mg/kg, dead animals - in all dead animals, cerebral edema and plethora of its membranes, pulmonary edema and congestion of internal organs were found. Two females had hemorrhages in the stomach, small and large intestines, one of them had necrosis of the epithelium of the intestinal mucosa and inflammatory Infiltration of the underlying areas. The cause of death of all dead animals is acute heart failure. Dose 4000 mg/kg, dead animals - in all dead animals, cerebral edema and plethora of its membranes were found. Most of the animals (80%) were found to have plethoric internal organs. Two males were diagnosed with pulmonary edema, and one female with emphysema. Histological examination of the lungs of one male

	showed edema and multifocal bronchopneumonia.

	More than 50% of the animals had intestinal hemorrhages, and the intestinal contents were watery. Histological examination of animal intestines showed atrophy of villi and crypts, vacuolization and necrosis of mucosal epithelial cells. Two males had hemorrhages in the stomach. The cause of death of all animals was acute heart failure.
Mass coefficients of internal organs	Dose 1000 mg/kg, males - an increase in the mass coefficients of the adrenal glands relative to the control group.
Evaluation of local tolerability (LIA)	*Routine necropsy:* Doses of 500, 1000, 2000 mg/kg - in all euthanized animals no macroscopically visible changes were found at the injection site. Dose 2000 mg/kg - in one male, a focus of fibroplasia was found in the tissues surrounding the injection site. Dose 3000 mg/kg - three animals had hemorrhagic impregnation of the tissues surrounding the caudal vein. Dose 3250 mg/kg - in two males, hemorrhagic impregnation was found in the tissues surrounding the injection site, in one of them a thrombus with vascularization was shown in the lumen of the tail artery. Dose 4000 mg/kg - planned necropsy was not performed due to the death of all animals in this group. Dose 3500 mg/kg - one female had hemorrhagic impregnation of the tissues surrounding the caudal vein. *Unplanned necropsy:* Dose 3250 mg/kg - in all animals, hemorrhagic impregnation of the tissues surrounding the caudal vein. Dose 3500 mg/kg - one male had a thickened caudal vein due to plethority. Dose 3750 mg/kg - in all animals, hemorrhagic impregnation of the tissues surrounding the injection site (caudal vein). One female also had a thickened vessel wall. Dose 4000 mg/kg - Half of the animals had hemorrhagic impregnation of the tissues surrounding the caudal vein, one female has plethoric caudal vein. One female has swelling of the vein wall, neutrophilic infiltration of the wall and perivascular region (vasculitis).

CONCLUSION

Study code: 3.28/21, Test system: Wistar rats, 5 males + 5 females in each group, route of administration – intravenous

Doses based active substance, mg/kg	Key results

0	**Lethality:** 0 % **Intoxication:** no lethal outcomes or signs of intoxication were recorded throughout the experiment **Pathomorphological changes:** Clinically significant No pathomorphological changes were found **LIA:** no changes at the injection site were found.
500	**Lethality:** 0 % **Intoxication:** no lethal outcomes or signs of intoxication were recorded throughout the experiment **Pathomorphological changes:** no pathomorphological changes were found **LIA:** no changes at the injection site were found.
1000	**Lethality:** 0% **Intoxication:** ruffled hair, inhibition. **Pathomorphological changes:** a single case of plethority of the spleen. **Organ Mass Ratios:** An increase in adrenal mass coefficients in males compared to controls. **LIA:** no changes at the injection site were found.
2000	**Lethality:** 0% **Intoxication:** ruffled hair, inhibition in females. **Pathomorphological changes:** no pathomorphological changes were found **LIA:** focus of fibroplasia in one male.
3000	**Lethality:** 0% **Intoxication:** inhibition of various severity, ruffled hair. **Body weight**: decrease in the body weight of the animals on day 2 of the experiment compared to the baseline values. **Pathomorphological changes:** no pathomorphological changes were found. **LIA:** hemorrhagic impregnation of the tissues surrounding the caudal vein (three cases).
3250 (males)	**Lethality:** 60%, predominantly on day 3 **Intoxication:** ruffled hair, inhibition of the general condition, in isolated cases shortness of breath, discharge from the nose and eyes, tremor. **Body weight**: decrease in body weight of males on day 2 of the experiment compared to baseline values. **Pathomorphological changes:** cerebral edema and plethora of its membranes, pulmonary edema and plethora of blood of internal organs. Hemorrhages in the stomach (2 cases), small and large intestines, necrosis of the epithelium of the intestinal mucosa. In a single case, an ulcer. **LIA:** hemorrhagic impregnation of tissues at the injection site, in a single case a blood clot with vascularization was found in the lumen of the tail artery.
3500	**Lethality:** 60% of males, predominantly on day 4 **Intoxication:** ruffled hair, inhibition, in isolated cases discharge from the nose and eyes. **Body weight**: decrease in body weight on day 2 of the experiment compared to baseline values. **Pathomorphological changes:** cerebral edema and cerebral hemorrhage

	of membranes, pulmonary edema and congestion of internal organs. Chronic lobular purulent bronchopneumonia (2 cases), extensive hemorrhages and focal pleurisy and adhesions of the caudal lobes of the lungs to the dorsal part of the pleura (1 case). **LIA:** hemorrhagic impregnation of tissues at the injection site (1 case), thickening of the caudal vein due to congestion (1 case).
3750 (females)	**Lethality:** 100%, predominantly on day 4 **Intoxication:** ruffled hair, inhibition, in isolated cases discharge from the nose and eyes. **Body weight**: decrease in body weight of females on day 2 of the experiment compared to baseline values. **Pathomorphological changes:** cerebral edema and plethora of its membranes, pulmonary edema and congestion of internal organs. Chronic lobular suppurative bronchopneumonia (2 cases), extensive hemorrhages, focal pleurisy and adhesions of the caudal lobes of the lungs to the dorsal part of the pleura (1 case). **LIA:** hemorrhagic impregnation of tissues at the injection site (1 case), thickening of the caudal vein due to full-bloodedness (1 case).
4000	**Lethality:** 100%, predominantly on days 3-4 **Intoxication:** inhibition of the general condition of various severity, ruffled hair, ptosis, decreased reaction to stimuli. **Body** weight: decrease in body weight of females on day 2 compared to the control group, decrease in body weight of males and females on day 2 of the experiment compared to baseline values. **Pathomorphological changes:** cerebral edema and plethora of its membranes. Most animals (80%) have plethora of internal organs. Pulmonary edema (1 case), emphysema (1 case). Edema and multifocal bronchopneumonia (1 case). More than 50% of the animals had hemorrhages in the intestines, the contents of the intestines were watery. Gastric hemorrhages (2 cases). **LIA:** hemorrhagic impregnation of the tissues surrounding the caudal vein. Edema of the vein wall, neutrophilic infiltration of the wall, and vasculitis (1 case).

SUMMARY

The picture of intoxication (inhibition of various severity, ruffled hair, bloody discharge from the nose and eyes) was observed when the test object was administered in doses of 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 3250 mg/kg, 3500 and 4000 mg/kg. Animal mortality was observed at doses of 3250 mg/kg, 3750 mg/kg, and 4000 mg/kg. The cause of death of all the dead animals was acute heart failure.

The maximum tolerated dose (MTD) for male rats was 3000 mg/kg, for females - 3500 mg/kg.

LD_{50} in males with intravenous administration - 3225±95.2 mg/kg, in females 3625±62.5 mg/kg. According to the classification of toxicity of substances with parenteral methods of administration by Sidorova K.K., the test object is assigned to the 6th class of low-toxic substances. According to the draft classification of chemicals proposed by Berezovskaya I.V., when administered intravenously, the object is assigned to the 4th class of low-toxic substances according to the draft classification GOST 12.1.007-76 (LD_{50} intravenous >700 mg/kg when administered intravenously to rats) and to hazard class 5 according to the draft OECD GHS. classification (LD_{50} intravenous > 700 mg/kg).

Keywords: Lithium ascorbate, acute toxicity, rats, intravenous administration

The report is presented on 285 pages, including 20 tables and 29 figures.

STUDY DATES

Study start date:	July 19, 2021
Start date of the experimental part:	August 10, 2021
Dates of dispensing of the test object:	August 10, 2021 August 13, 2021 August 16, 2021 August 18, 2021 September 22, 2021 September 27, 2021
End date of the experimental part:	October 11, 2021
Date of preliminary report:	October 25, 2021
Study completion date:	November 8, 2021

LIST OF EXECUTORS

Study Leader	[Signature] 08.11.2021 signature, date	A.V. Popova (abstract, sections 1, 2, summary, conclusions)
Head of the Pharmacist Service	[Signature] 08.11.2021 signature, date	N.A. Kishchenko (subsection 1.1)
Veterinarian of the vivarium	[Signature] 08.11.2021 signature, date	D.Yu. Akimov (subsection 1.2)
Head of the Laboratory Diagnostics Department	[Signature] 08.11.2021 signature, date	Y.A. Gushchin (subsections 1.4.6, 1.4.7, 2.3

STUDY DECLARATION OF GLP COMPLIANCE

FROM THE STUDY LEADER

Study Code:	3.28/21
Study title:	Study of the toxic properties of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats
Test object:	Lithium Ascorbate, substance (Normopharm LLC, Russia)

This study was carried out in accordance with the standard operating procedures of the institution, and the Study plan № 3 and Amendments to the Study plan №№1-3 mutually agreed with the Sponsor (Normopharm LLC, Russia).

The study complies with the GLP requirements, except for the fact that no identification, purity, and stability studies of the test object have not been carried out by the research institution.

Ref. №	*Details/Aspects*	*Date of signature by the Study leader*	*Date of signature by the Sponsor's representative*
1	Study plan №3	19.07.2021	25.06.2021
2	Amendment №1	16.08.2021	16.08.2021
3	Amendment №2	15.09.2021	15.09.2021
4	Amendment №3	27.09.2021	27.09.2021

There were no deviations from the Study Plan that would affect data interpretability or the scientific integrity and results of the study.

I, the undersigned, hereby confirm that I take overall responsibility for the technical conduct of the study; analysis, interpretation, documentation and presentation of results, as well as archiving of the study-related materials.

The objectives set out in the study plan have been achieved. There were no unforeseen circumstances that could affect the quality or integrity of the study.

This report presents the results reliably. I am fully responsible for the accuracy of the data obtained, as well as the confidentiality of the preclinical study.

I guarantee that after the study completion, the study plan, the final report, source data and all relevant documentation will be transferred to the archives of the research institution.

Study leader

A.V. Popova		08.11.2021
/Name/		/Date/

STATEMENT BY THE MANAGEMENT OF THE RESEARCH INSTITUTION ON THE PROVISION OF RESOURCES TO CONDUCT THE STUDY IN ACCORDANCE WITH THE GLP PRINCIPLES, REGULATORY REQUIREMENTS AND STANDARDS FOR THE ETHICAL HANDLING OF ANIMALS

Study code:	3.28/21
Study title:	Study of the toxic properties of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats
Test object:	Lithium Ascorbate, substance (Normopharm LLC, Russia)

The management of the research institution shall ensure that the following has been provided for the proper conduct of the study:

- availability of a sufficient number of qualified and experienced personnel with a clear understanding of their responsibilities, as confirmed by training data;
- equipping the research institution with the necessary equipment, facilities and materials;
- availability of a quality service responsible for the quality assurance system;
- availability of approved standard operating procedures, as well as access to them by all personnel involved in the conduct of the study;
- appointment of a study leader in accordance with the established procedure, who has qualifications appropriate to the study objectives;
- interaction of the study leader, employees of quality service and personnel involved in the study.

Compliance with the Principles of Good Laboratory Practice

This study was carried out in accordance with the principles of GLP OECD (GOST 33044-2014 “Principles of Good Laboratory Practice”; Decree of the EEC Council № 81 “On Approval of the Rules of Good Laboratory Practice of the Eurasian Economic Union in Circulation of Medicines” dated 03.11.2016). The manipulations were performed in accordance with the standard operating procedures of the institution and Study plan № 4.

Regulatory Compliance

The design of this study was based on the selection of the study goal, in accordance with the regulatory legal acts and guidelines laid down in “Regulatory Standards” section of this Report.

Compliance with the Standards of Ethical Handling of Animals

This study was reviewed at a meeting of the Bioethics Commission for compliance of the draft study with the “Three R’s” principles and Directive 2010/63/EU. The study was approved for conduct (№ BEC 3.42/21 dated 18.06, 2021, 7 persons voted).

General Director of CJSC “Saint Petersburg Institute of Pharmacy”

V.G. Makarov	[Signature]	08.11.2021
/Name/	*/Signature/*	*/Date/*

STATEMENT OF THE QUALITY SERVICE ON CONDUCTING AND DOCUMENTING THE INSPECTION OF THE KEY STAGES OF THE STUDY

Study code:	3.28/21
Study title:	Study of the toxic properties of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats
Test object:	Lithium Ascorbate, substance (Normopharm LLC, Russia)

The Quality Service conducted and documented all stages of the study inspection. The results were reported to the study leader and the management of the research institution.

The study was inspected to ensure that the procedures and manipulations performed were in accordance with the standard operating procedures of the institution, the study plan, and the regulatory requirements of the Good Laboratory Practice standards.

The final study report was reviewed by a quality officer and found to be an accurate statement of the data obtained and the procedures applied. The results presented in the final report accurately and fully reflect the data obtained during the study.

The conclusion of the quality service on this study is an Annex to the final report.

S.S. Sapynov	[Signature]	08.11.2021
/Name/	*/Signature/*	*/Date/*

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LIST OF ABBREVIATIONS AND ACRONYMS

In this R&D report, the following abbreviations and acronyms are used:

ANOVA	ANalysis Of VAriance, analysis of variance
BEC	Bioethics Commission
CJSC	Closed Joint-Stock Company
EAEU/EEC	Eurasian Economic Union
F	Females
FL	Federal Law
GIT	Gastrointestinal tract
GOST	State Standard
I/V	Intra
INN	International Non-proprietary Name
JSC	Joint-Stock Company
GLP	Good Laboratory Practice
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
LD_{50}	Mean lethal dose
LIA	Local irritant action
LLC	Limited Liability Company
M	Mean
M	Males
N	Number of observations
NPO	Research and Production Association
OECD	Organisation for Economic Cooperation and Development
pH	pondus Hydrogenii (Hydrogen value)
R&D	Research and development work
RF	Russian Federation
SanPiN	Sanitary Rules and Regulations
SEM	Standard error of the mean
SOP	Standard Operating Procedure
SP	Study plan

INTRODUCTION

The test object is Lithium Ascorbate, substance (Normopharm LLC).

Lithium ascorbate is a highly absorbable and low-toxic organic lithium salt [1]. Lithium salts are widely used as normothymics in various affective disorders [2]. Lithium ions have a significant effect on the homeostasis of acetylcholine, enkephalins, catecholamines, serotonin, and other neurotransmitters [3]. Lithium (primarily as lithium carbonate) for the treatment of bipolar disorder or inhibition [4, 5] is used in doses in the hundreds of milligrams. Such doses may lead to severe adverse effects during therapy (renal pathology, teratogenesis). Compared to lithium carbonate therapy, lithium ascorbate has also shown efficacy in ultra-low doses [1].

This study aimed at evaluating the toxic properties and local irritant action of substance Lithium Ascorbate following a single intravenous administration to sexually mature rats, is part of the complex of preclinical studies required for the Recording of the drug in the Russian Federation (RF) [6, 7].

The study was carried out with the engagement of employees of the necessary departments [Appendix A], the approved study plan, Amendments №№. 1-3 to the Study Plan [Appendix B] and approved by the bioethics commission [Appendix C].

The information obtained in the study did not duplicate the results of previous studies.

STUDY GOAL AND OBJECTIVES

Study goal:

Study of the toxic properties of substance Lithium Ascorbate (Normopharm LLC) following a single intravenous administration to sexually mature rats.

Study objectives:

1 study of toxic properties of the test object with analysis of the clinical picture of intoxication;

2 evaluation of local tolerability;

3 determination of the mean lethal dose (LD_{50}) of the test object.

1 Materials and methods

1.1 Study objects

Information on the test object and vehicle is given in Tables 1.1.1.1 and 1.1.2.1.

1.1.1 Test object

Table 1.1.1.1 – Test object

INN:	Lithium Ascorbate
Study object code:	T-3.28/21
Manufacturer:	Normopharm LLC
Form:	Substance
Batch:	Batch number is not provided by the Sponsor (study protocol №120a/21, Annex D)
Manufacturing date:	15.12.2020
Shelf life:	15.12.2022
Storage conditions:	In a place protected from light at a temperature of +2°C to +8°C. It may be stored for a short period of 3-5 days at room temperature.

1.1.2 Control substance

Table 1.1.2.1 – Control substance

INN:	Sodium chloride, solution for infusions 0.9%
Manufacturer:	Gematek LLC, Russia
Study object code:	M-3.28/21
Dosage form:	Solution for infusions, 250 ml vials
Series:	21290421
Shelf life:	31.03.2024
Storage conditions:	At temperatures not above 25°C

The documents of the pharmacist service are given in Appendix D.

The research institution has not carried out any identification, purity and stability studies of the test object. These values were determined by the Sponsor according to standard methods. The Sponsor of the study is responsible for the reliability of the submitted data on the identification, purity and stability of the test object.

1.2 Animals

Species:	Wistar rats
Sex:	Males and females (nulliparous and non-pregnant)
Source:	JSC “NPO “HOUSE OF PHARMACY”
Animal birth certificates:	№2.1-30.06/21 dated 30.06.2021 №2.1-31.08/21 dated 31.08.2021 [Annex E]
Rationale for the selection of animal species/lines	Wistar rats are successfully used in toxicological experiments [6].

Number of groups (according to the study plan):	Total number of groups: 8 Control substance intravenously [M] - group №1 Test object intravenously [T] - groups №№ 2-8
Number of rats/group (in accordance with the study plan):	10 rats (5 males/5 females) / group
Total number of rats:	80 rats (40 males, 40 females). Additional 10 male and 10 female rats were available if they needed to be replaced during the adaptation period. After the end of the adaptation period, the uninvolved animals served as sentinel animals, i.e. they were kept in the same conditions as the experimental animals until the end of the experimental part of the study work. The sentinel animals not involved in the experiment were returned to the veterinary service.
Age at the start of the test object administration:	8-10 weeks
Range of body weight values at the start of the experiment, M±SEM:	males – 196.6±2.06 g, females – 176.6±1.40 g
Quality category of animals:	We used animals free of: *Streptococcus sp.-haemolyticus, Streptococcus pneumoniae, Pasteurella pneumotropica, Mycoplasma pulmonis, Salmonella spp., Clostridium spp.,* endo- and ectoparasites (mites, helminths and protozoa). Health passport № 2.1/2021.
Identification:	Each animal within the group was marked at the tail using a permanent marker. In accordance with the mark, each animal selected for the study was assigned an individual number. This number consisted of two parts: the 1st part was the number of the group, and the 2nd part was the serial number of the animal in the group. Numbering within the group was consecutive, first, males, then females were numbered.

The labeling of the cage included the number of the bioethics commission's report (study code), cage number, species, line, sex and number of animals, dates of formation of the groups, the beginning and end of the experiment, the number of the experimental group, number of animals, the code, the code, route of administration and the dose of the test object, surname of the study leader.

Adaptation and selection of animals:

Prior to the study, animals were kept in breeding cells.

When the animals were transferred to the experiment, a lot of animals was clinically examined, and the "Act of Acceptance and Transfer of Laboratory Animals" [Annex E] was completed. Clinically healthy animals were transferred.

The adaptation of rats in group cages was 5 to 10 days. During this period, the clinical condition of the animals was monitored every day by visual examination. During the adaptation period, no abnormalities in the clinical condition were found.

Group allocation:

To exclude the influence of the investigator's preferences on the formation of experimental groups, animals were selected with the method of modified block randomization [8]. To do this, all animals submitted to the study were randomly placed in the cages of the randomization block (the number of cages of the randomization unit is a multiple of the number of groups in the experiment). Then, using a random number generator, a list of data was obtained, containing the numbers of the cages with animals and the corresponding numbers of the groups where the animals were subsequently allocated [9] [Annex F].

The animals were kept under standard conditions in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes [10].

Accommodation system: Indoor air control in compliance with environmental parameters.

Temperature/ humidity:	18-26°C, 46-65%. No deviations from the established environmental parameters occurred during the adaptation period and during the experiment.
Ammonia and carbon dioxide levels:	NH_3 = 0 mg/m^3, CO_2= 0 vol.% (measured on May 24, 2021)
Air exchange mode:	About 10-15 room volumes per hour
Light regime:	12 dark hours/12 light hours
Cages:	The rats were kept in standard plastic cages, on a bedding; The cages were covered with steel lattice covers with a food hopper. The floor area per animal complied with regulatory standards [10].
Allocation:	Rats were kept in groups of 5 animals of the same sex.
Feed:	The animals were fed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union dated 22 September 2021 for the protection of animals used for scientific purposes. Feed for keeping laboratory animals, prepared according to GOST 34566-2019 “Complete feed for laboratory animals. Specifications” were given to the food hopper of the steel lattice lid of the cage. Declaration of Conformity - ROSS RU D-K.i.AA80.B.01275/19 valid until 10.07.2022.

Water: The animals were given purified water, standardized for organoleptic properties, pH, dry residue, reducing substances, carbon dioxide, nitrates and nitrites, ammonia, chlorides, sulfates, calcium and heavy metals on the basis of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, for water objects, drinking water and drinking water supply to the population, atmospheric air, soils, living quarters, operation of industrial and public premises, organization and implementation of sanitary anti-epidemic (preventive) measures". Water in standard drinking bowls with steel spouts was given *ad libitum*.

1.3 Method of administration and dose selection

1.3.1 Method and duration of administration

The test object was administered intravenously (i/v) to the animals. In the study of acute toxicity, the test object and the control substance were administered once (if necessary, in fractions) with further observation of the animals for 14 days [11].

1.3.1 Selection and calculation of doses

In this study, in accordance with the Sponsor's recommendation, the test object was to be administered in the following doses: 125 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg.

The experiment was carried out in stages and began with the object administration in a dose of 500 mg/kg. After the administration of the test object, the condition of the animals was satisfactory, no death was observed, based on which doses of 1000 mg/kg and 2000 mg/kg were selected for administration. Doses of 1000 mg/kg and 2000 mg/kg did not cause lethal effects, based on which the range of studied doses was changed for the calculation of LD_{50}: instead of the planned doses of 125 mg/kg and 250 mg/kg, the animals were administered with doses of 3000 mg/kg and 4000 mg/kg (in accordance with Amendment № 1 of 16.08.2021) [Annex B]. As a result of the administration of a dose of 4000 mg/kg, the mortality rate was 100%, and the administration of a dose of 3000 mg/kg was not accompanied by the death of animals. Based on these data, it was decided to introduce two additional groups for the calculation of LD_{50} (in accordance with Additional Agreement № 1 dated 15.09.2021 and Amendment № 2 dated 15.09.2021), the animals of group № 7 received the test object in a dose of 3500 mg/kg. Since the

mortality rate in males was 60%, and no lethal effects were observed in females, the dose for males in group № 8 was 3250 mg/kg. and for females 3750 mg/kg (in accordance with Amendment № 3 of 27.09.2021).

Thus, male rats were administered the test object once intravenously, if necessary, in fractions, at doses of 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 3250 mg/kg, 3500 mg/kg and 4000 mg/kg, and female rats in doses of 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 3500 mg/kg. 3750 mg/kg and 4000 mg/kg.

The control substance was administered to the animals of group № 1 once, fractionally, in an amount equivalent to the maximum dosing volume of the test object.

1.3.2 Dosing procedure

The test object (as a solution) and the control substance were administered to the animals once intravenously, if necessary, in fractions, using catheters and syringes.

The test object was administered to animals once, doses of 3000 mg/kg, 3250 mg/kg, 3500 mg/kg, 3750 mg/kg and 4000 mg/kg were administered in fractions, in two equal parts, using catheters and syringes, with an interval between injections of at least 30 minutes.

1.4 Methodology

1.4.1 Study design

The total number of animals involved in the experiment is 80 Wistar rats (40 males and 40 females). The characteristics of the experimental groups and the design of the experiment are presented in Tables 1.4.1.1 and 1.4.1.2.

Table 1.4.1.1 - Characteristics of experimental groups

<table>
<tr><th rowspan="2">Group number</th><th colspan="2">Number of animals</th><th rowspan="2">Test object [study object code]</th><th rowspan="2">Dose, mg/kg</th><th rowspan="2">Euthanasia day</th></tr>
<tr><th>females</th><th>males</th></tr>
<tr><td>1</td><td>5</td><td>5</td><td>Control substance [M-3.28/21]</td><td colspan="2">0</td><td rowspan="10">14 days after administration (on day 15 of the experiment)</td></tr>
<tr><td>2</td><td>5</td><td>5</td><td rowspan="9">Test object Lithium Ascorbate [T-3.28/21]</td><td colspan="2">4000 [a]</td></tr>
<tr><td>3</td><td>5</td><td>5</td><td colspan="2">3000 [a]</td></tr>
<tr><td>4</td><td>5</td><td>5</td><td colspan="2">500</td></tr>
<tr><td>5</td><td>5</td><td>5</td><td colspan="2">1000</td></tr>
<tr><td>6</td><td>5</td><td>5</td><td colspan="2">2000</td></tr>
<tr><td>7</td><td>5</td><td>5</td><td colspan="2">3500 [b]</td></tr>
<tr><td rowspan="2">8</td><td rowspan="2">5</td><td rowspan="2">5</td><td>males</td><td>females</td></tr>
<tr><td>3250 [c]</td><td>3750 [c]</td></tr>
</table>

The test object was administered to all groups at the same concentration - 125 mg/ml.

Dosing volumes of the test object in different groups are presented in Table 1.3.3.1.

Table 1.4.1.2 – Dosing volumes of the test object

Dose of the test object	Dosing volume	Concentration
500 mg/kg	4 ml/kg	125 mg/ml
1000 mg/kg	8 ml/kg	
2000 mg/kg	16 ml/kg	
3000 mg/kg [a]	24 ml/kg	
3250 mg/kg[c]	26 ml/kg	
3500 mg/kg [b]	28 ml/kg	
3750 mg/kg[c]	30 ml/kg	
4000 mg/kg [a]	32 ml/kg	

Remarks

1 - [a] - doses are given on the basis of Amendment № 1 of 16.08.2021;

2 - [b] - doses are given on the basis of Amendment № 2 of 15.09.2021;

3 - [c] - doses are given on the basis of Amendment № 3 of 27.09.2021.

Remarks

1 - [a] - the dose is given on the basis of Amendment № 1 dated 16.08.2021;

2- [b] - the dose is given on the basis of Amendment № 2 of 15.09.2021;

3 - [c] - the dose is given on the basis of Amendment № 3 of 27.09.2021

Table 1.4.1.2 – Manipulation schedule

Manipulation	Experiment Days
Body weight recording	Days 1, 2, 8, 15
Administration of the test object and control substance	Day 1
Clinical examination	Pre-dosing, days 2, 8, 14
Clinical observation	Days 1-15
Euthanasia of all animals	Day 15

1.4.2 Feed deprivation

The animals were deprived of feed 16 hours before dosing, each body weight record and euthanasia. Access to water was ad libitum throughout the experiment.

1.1.1 **Body weight recording**

Body weight data on day 15 of the experiment were used to calculate the percentage ratio of the mass of internal organs to body weight. Source data are presented in source charts [Appendix G].

The procedure of weighing rats was carried out on an electronic balamce Vibra AJ-1200CE (Shinko Denshi, Japan). The maximum weighing limit is 1200 g, the minimum weighing limit is 0.5 g. Calibration mark is 0.1 g. Accuracy class is 2 [Appendix B].

1.4.3 Recording of the timing of the development of intoxication and clinical examination of animals

1.4.3.1 Recording of the timing of the development of intoxication

The animals were continuously monitored prior to administration, for 30 minutes after dosing of the last portion of the test subject at fractional administration, then hourly for 4 hours, then after 24 hours, and then daily for 15 days.

The following was recorded:

2 Behavior: distress/agitation;

3 Response to stimuli: decrease/increase;

4 Skin: redness / paleness / cyanosis / jaundice;

5 Mucous membranes: redness/pallor/cyanosis/jaundice;

6 Discharge: from the eyes / from the nose / from the anus / from the urethra;

7 Muscle tone: decrease/increase;

8 Motor coordination disorders: ataxia/hyperkinesis;

9 Dyspnea;

10 Changes in the site of administration;

11 Death.

11.4.3.1 Clinical examination

The animals were clinically examined prior to administration, then on days 2, 8 and 14 of the experiment. A detailed examination of the animal was performed in the cage, in the hands and in the open area. The manifestation and severity, where acceptable, of signs of intoxication were recorded.

1) Examination in the cage:

- Behavior: normal/distress/agitation;
- Attitude towards other animals: normal/ aggression.

2) Examination when taking up an animal:

- Response to stimuli: normal/decrease/increase;
- Body condition: normal / dystrophic / obese;
- Muscle tone: normal/decreased/increased;
- Hair: normal (smooth, shiny) / ruffled / hair loss / dull / dirty / discoloration.
- Skin:
- Turgor: normal/reduced;
- Color: normal / redness / paleness / cyanosis / jaundice / hemorrhage;
- Integrity: normal (not impaired)/abrasions/cracks/wounds;
- Palpable masses.
- Mucous membranes:
- Color: normal / redness / paleness / cyanosis / jaundice;

Impaired integrity.

- Eyes: normal/exophthalmos (bulging eyes)/impaired integrity/discharge;
- Nasal cavity: normal / serous discharge / purulent discharge / bloody discharge;
- Oral cavity: normal/drooling.

3) Outdoor examination:

- Position of the body in space: normal / forced lying down / forced wandering in a circle / forced movement forward and backward / forced desire to lie on one side;
- Impaired motor coordination: normal/ataxia/hyperkinesis;
- Breathing type: normal / thoracic /abdominal /dyspnea;
- Bowel movements: normal / diarrhea / presence of blood in the stool / change in stool color;
- Urination: normal/discoloration;

- Changes in the site of administration.

1.4.5 Euthanasia

On day 15 of the experiment, the animals were euthanized with CO_2 followed by exsanguination from the heart cavities. In accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes of 22 September 2010 [10], this type of euthanasia of animals is accompanied by a minimum of pain, suffering and distress and is carried out by competent personnel.

1.4.6 Pathomorphological examination

The post-mortem examination included a macroscopic evaluation of the animals euthanized according to the schedule, as well as a pathomorphological examination of the corpses of animals that died during the experiment [12].

Necropsy was performed under the direct supervision of a pathologist. After euthanasia, the animals were carefully examined for external pathological signs. Evaluation of the condition of thoracic and abdominal cavity and a macroscopic examination of the internal organs were carried out. Similar examinations were carried out on dead animals with the completion of a necropsy map [Appendix K].

Organs extracted during necropsy were weighed, and paired organs were weighed together. This value was used to calculate the percentage ratio of organ mass to body weight.

The procedure of weighing the internal organs was carried out on the electronic balance “Adventurer” RV 214 (OHAUS, China). The maximum weighing limit is 210 g, the minimum weighing limit is 0.001 g. Calibration mark is 0.001 g. Accuracy class is 1 [Appendix B].

List of organs to be weighed:

- Heart
- Lungs with trachea
- Thymus
- Liver
- Spleen
- Kidneys
- Adrenal glands
- Cerebrum
- Testes/ovaries

Organ collection

During necropsy, the organs (organ fragments) listed below were taken and embedded in 10% pH-neutral formalin.

The organs have been archived and will be handed over to the Sponsor upon request.

- Lungs with trachea
- Heart
- Thymus
- Liver
- Liver
- Kidneys
- Adrenal glands
- Spleen
- Stomach
- Small intestine
- Large intestine
- Cerebrum
- Testes/ovaries
- Caudal vein

Histology

Histological examination was performed if macroscopic changes in internal organs during necropsy were found, including animals that died on the first day after the administration of the test object (only the tissue of the altered organ was examined to clarify the diagnosis). Lungs, spleens, testes, gizzards, intestinal fragments, and liver fragments from several animals were taken for histological examination.

For histological examination, the material was fixed in a 10% solution of neutral formalin for 24 hours, after which it was embedded paraffin according to the generally accepted method [13]. Then sections with a thickness of 5-7 μm were made, which were stained with hematoxylin and eosin. The analysis of histological specimens was carried out using a light-optical microscope Axio Scope A1 ZEIZZ (Carl Zeiss MicroImaging GmbH, Germany) at a magnification of 50, 100, 400, 1000. Microphotography was made with a digital camera AxioCam ICc1 (Carl Zeiss MicroImaging GmbH, Germany) and ZEN 2012 software.

1.4.7 Evaluation of local tolerability

To evaluate the local tolerability of the test object, the condition of the skin at the injection site was evaluated during clinical examinations and observations, and the condition of the skin and adjacent soft tissues at the injection site was visually evaluated during the necropsy procedure. If abnormalities were found during the macroscopic examination, a routine histological examination of the tissues in direct contact with the test object was performed.

1.4.8 Data analysis

Descriptive statistics was applied to all data: the data were checked for compliance with the normal distribution law using the Shapiro-Wilk's W test. For data with a normal distribution, the mean value and the standard error of the mean were calculated, which, together with the value of n (number of cases), are presented in the summary tables. To evaluate data with signs of normal distribution, a one-factor analysis of variance was used (ANOVA), if a significant effect of the studied factor was found, subsequent intergroup comparisons (*post hoc* analysis) were made using the Tukey's test analysis. Differences were determined at the significance level of $p<0.05$.

Statistical analysis was performed using licensed software Statistica 10.0 (StatSoft, USA).

The LD_{50} was calculated using the Bliss-Prozorovsky method [14].

1.4.9 Study quality assurance and control

The quality service of the research institution carried out [Appendix K]:

- verification of the study plan
- checking the study plan - schedule
- incoming audit of the preclinical study
- audit of the experimental part of the study
- checking the chronology of the study and the completeness of the study report
- verification of the final study report.

2 Study results

The study of toxic properties with an evaluation of the local irritant effect of lithium ascorbate, substance (Normopharm LLC, Russia), was carried out following a single intravenous administration into male and female outbred rats. The data obtained during the experiment are presented in full in the source charts [Appendix G].

2.1 Toxicometry

2.1.1 Lethality

During the experiment, the death of 11 males and 10 females was recorded. Lethal effects based on the results of 48 hours of observation are presented in Table 2.1.1.1, mortality for 14 days of the experiment is presented in Table 2.1.1.2. Animal deaths were recorded in groups № 2, № 7 and № 8 (doses of 3250 mg/kg and higher).

In the groups of animals that received the test object in doses of 3250 mg/kg, 3500 mg/kg, 3750 mg/kg and 4000 mg/kg, mortality was delayed (48 hours after the administration of the test object), data on the timing of animal death are presented in Tables 2.1.1.3 and 2.1.1.4.

Doses of 3,000 mg/kg for males and 3,500 mg/kg for females are considered to be the

maximum tolerated doses (the maximum doses studied in which no animal death was observed). Intravenous LD_{50} in males - 3225±95.2 mg/kg, in females - 3625±62.5 mg/kg [17]. The tested object is classified as Class 6 of low-toxic substances. According to the draft classification of chemicals proposed by Berezovskaya I.V. [17], when administered intravenously, the object is assigned to the 4th class of low-toxic substances according to the draft classification GOST 12.1.007-76 (LD_{50} >700 mg/kg when administered intravenously to rats) [15, 17] and to hazard class 5 according to the draft classification of the GHS OECD (intravenous LD_{50} > 700 mg/kg) [16, 17].

2.1.2 Picture of intoxication. Clinical examination

Dose 500 mg/kg. No deaths or signs of intoxication were recorded throughout the experiment.

Dose 1000 mg/kg. Within the first hour after administration, ruffled hair was observed in most of the animals, inhibition was also recorded in one female (Table 2.1.2.1), then the condition of the animals gradually returned to normal. On the second day, the condition of all the animals was satisfactory, except for one male with ruffled hair, by the third day, the condition of this animal was completely normal.

Dose 2000 mg/kg. Within the first hour after administration, ruffled hair was observed in the animals, and inhibition was recorded in most of the females (80%) (Table 2.1.2.2). By the second hour after administration, the condition of all animals was completely normal.

Dose 3000 mg/kg. In the first 3 hours of observation, the pattern of intoxication was manifested as ruffled hair and inhibition (Table 2.1.2.3). In the following days, in most animals, the severity of toxic manifestations decreased, intoxication manifested itself only in ruffled hair. By the 6th day after administration, the condition of all animals was satisfactory.

Dose 3250 mg/kg (males). On day 1, inhibition and ruffled hair were observed in all animals. The picture of intoxication gradually increased the most intensive signs were found on the second day after administration. Dyspnea, nasal and eye discharge were reported in animals (Table 2.1.2.4). By the 10th day of observation, the condition of the surviving animals was completely normal.

Dose 3500 mg/kg. In the first 24 hours after administration, inhibition and ruffled hair were observed in the animals, and ptosis was recorded in 40% of rats. From the second day of observation, inhibition of various degrees of severity, ruffled hair, in isolated cases discharge from the eyes and nose, pallor of the skin were noted in the animals. The maximum intoxication occurred on the 3rd day after administration of the object (Table 2.1.2.5). In the survived females, signs of intoxication persisted until the 6th day after administration, in the survived males, signs

of intoxication persisted until the end of the experiment.

Dose 3750 mg/kg (females). In the first 24 hours after administration, all animals showed signs of intoxication such as inhibition and ruffled hair. On the second day of administration, one female rat had tremors. Most intensive intoxication occurred on the 3rd day after the introduction of the object (Table 2.1.2.6), inhibition, ruffled hair, discharge from the nose and eyes, and in a single case ataxia, were observed in the animals. By the 7th day of the experiment, the mortality rate was 100%.

Dose 4000 mg/kg. In the first 4 hours after administration, the animals were found to have ruffled hair, inhibition of various severity, ptosis, and an increased response to stimuli was recorded in females (Table 2.1.2.7). Signs of intoxication persisted until the death of the animals. By the 5th day of the experiment, the mortality rate in the group was 100%.

2.2 Effect of a single intravenous administration of the test object on the body weight of animals

Tables 2.2.1 and 2.2.2 provide the body weights of male and female rats following intravenous administration of the test subject. The data corresponded to the normal distribution law. Since animal mortality was observed in the groups that received the substance at doses of 3250 mg/kg and higher, the data obtained on days 1 and 2 of the experiment for all groups were processed at the first stage (analysis of variance with repeated measurements). Further, the groups in which there was death were excluded from the treatment, and using analysis of variance with repeated measurements, the body weight for the entire period of the experiment was estimated for the groups 0-3000 mg/kg. When processing the data obtained in the first two days of the experiment, a significant effect of the factor “repetition of measurements” ($p<0.05$) for male and female rats was found, the “group” factor was not significant for males ($p>0.05$). but significant for females ($p<0.05$). Subsequent intergroup comparison using the Tukey test showed a significant reduction in body weight relative to baseline values for groups of animals treated with the substance in doses of 3000 mg/kg and higher (Tables 2.2.1 and 2.2.2). The body weight of females that received the test object in a dose of 4000 mg/kg on the second day was significantly different from that of the control group (Tukey’s test, $p<0.05$). Processing of data from groups 0-3000 mg/kg (days 1-15 of the experiment) showed the importance of the factors “group” and “repeatability of measurements” for the body weight of animals. In all groups, positive body dynamics was observed. On days 8 and 15 of the experiment, the body weight of males significantly increased compared to the baseline one in the control group and in the groups that received the substance in doses of 500 - 2000 mg/kg (Tukey’s test, $p<0.05$). In the 3000 mg/kg group of males, a similar trend was observed, which did not reach statistical significance. In

females of the control group and in the groups that received the substance in doses of 500 - 3500 mg/kg, by day 15 of the experiment, body weight increased compared to the baseline, this increase reached statistical significance in the groups received the substance in doses of 500, 1000, 2000 and 3500 mg/kg.

2.3 Pathomorphological examination data

2.3.1 Results of pathomorphological examination of animals with unplanned necropsy

In all the dead animals of groups № 2, № 7 and № 8, cerebral edema and plethora of its membranes were found (Table 2.3.1.1, Figures 2.3.1.1-2.3.1.3), in most of dead animals (90%) of these groups, plethora of internal organs was shown. Pulmonary edema was found in all animals of groups 7 and 8, as well as in two animals of group 2 (Figures 2.3.1.4-2.3.1.6). In isolated cases, pulmonary hemorrhages were found (Figure 2.3.1.6). Histological examination of the lungs found chronic lobular purulent bronchopneumonia in two males of group № 7 (dose 3500 mg/kg) and one male of group № 2 (dose 4000 mg/kg). One female in group 2 was found to have emphysema.

Gastric hemorrhages were found in all dead males of group 8 (dose 3250 mg/kg) and two females of group 8 (dose 3750 mg/kg), as well as in two animals of group 2 (dose 4000 mg/kg), and ulcers were found in one male during the examination of the stomach. The gastric mucosa of a male rat from group 2 was loose, and that of one female of group 2 was plethoric.

Intestinal hemorrhages were observed in all dead males of group 8 (dose 3250 mg/kg), in two females of group 8 (dose 3750 mg/kg), as well as in half of the animals of group 2 (Figures 2.3.1.11 and 2.3.1.12). In several animals of group 2 (dose 4000 mg/kg), the intestinal contents were watery. Histological examination of the intestines of group 2 animals found atrophy of villi and crypts, vacuolization and necrosis of mucosal epithelial cells (Figures 2.3.1.13 and 2.3.1.14). In one female rat, the described changes were combined with pronounced reactive inflammatory infiltration of the areas subject to necrosis.

In group 8, one male had a variegated liver. Histological examination of the liver found plethora of the organ and diffuse small-droplet fatty dystrophy.

Changes in the injection site were also found in the dead animals (see section “Results of the evaluation of local tolerability”).

The immediate cause of death of all dead animals in groups № 2, № 7 and № 8 was acute heart failure.

2.3.2 Results of pathomorphological examination of animals with planned necropsy

Planned necropsia in group 5 showed an enlarged spleen in one female (Figure 2.3.2.1, Table 2.3.2.1), and histological examination of the spleen showed its plethora. In the rest of the

euthanized animals of this group, no macroscopically visible changes were found in the examined organs (Figure 2.3.2.2).

One male of group 1 was found to have a decrease in testicles (Figure 2.3.1.3). Histological examination of the testicles showed degeneration of the spermatogenic epithelium (Figure 2.3.1.4). According to the literature, it is known that such changes can occur spontaneously in rats [18]. The fact that the found changes occurred in a single case, in the group that received saline, suggest that the change was spontaneous.

In the rest of the euthanized animals, no macroscopically visible changes were found in the examined organs (Figures 2.3.1.5-2.3.1.8).

2.3.3 Mass coefficients of internal organs of experimental animals

Tables 2.3.3.1 and 2.3.3.2 showed the mass coefficients of the internal organs of experimental animals. The data corresponded to the normal distribution law. Univariate analysis of variance found the effect of the "group" factor on the mass coefficients of the heart, brain and adrenal glands of males, as well as on the mass coefficients of the kidneys of females (ANOVA, $p<0.05$). In male rats treated with the object in a dose of 1000 mg/kg, a statistically significant increase in the mass coefficients of the adrenal glands compared to the control group was found (Tukey's test, $p<0.05$). The values exceeded the upper limit of the reference intervals [16]. Also, in males of this group, a significant decrease in the mass coefficients of the heart was found, but these changes did not go beyond the reference values for animals of this weight [19]. In addition, the evaluation of the absolute masses of the heart did not show the influence of the group factor (ANOVA, $p>0.05$, Table 2.3.3.3). Therefore, the statistically significant differences found can be considered a variant of individual variability in animals, and not considered clinically significant. An intergroup comparison of data on the mass coefficients of the male brain did not establish any differences from the control group. In female rats that received the test object in doses of 2000 mg/kg and 3000 mg/kg, the mass coefficients of the kidneys were significantly lower than in the control group, but these changes were also within the reference values [19]. The assessment of the absolute masses of the organ did not show the influence of the "group" factor (ANOVA, $p>0.05$, Table 2.3.3.4). On this basis, the statistically significant differences found are not considered clinically significant.

2.3.4 Results of evaluation of local tolerability

Routine necropsy

Hemorrhagic impregnation of the tissues surrounding the caudal vein was found in three animals of group 3 (dose 3000 mg/kg), one animal of group 7 (dose of 3500 mg/kg) and two animals of group 8 (dose of 3250 mg/kg) (Figures 2.3.4.12.3.4.3, Table 2.3.4.1). In one male of

group 8, a blood clot with vascularization was also found in the lumen of the tail artery (Figure 2.3.4.2)

In one male of group 6 (dose 200 mg/kg), a focus of fibroplasia with an accumulation of hemosiderophages in its center was found in the tissues surrounding the injection site (Figure 2.3.4.4). The described microscopic changes characterize the process of arrangement at the site of the former hemorrhage.

Unplanned necropsy

Hemorrhagic impregnation of the tissues surrounding the caudal vein was shown in all dead animals of group 8 (dose 3250 mg/kg for males and 3750 mg/kg for females) and half of the animals of group 2 (dose 4000 mg/kg) (Figures 2.3.4.5-2.3.4.7, Table 2.3.4.1), and congestion of the caudal vein was also found in one female of group 2.

Histological examination of the caudal vein of a female rat of group 2 (dose 4000 mg/kg) found hemorrhagic impregnation of the tissues surrounding the vessel, as well as edema of the vein wall, neutrophilic infiltration of the wall and perivascular region (vasculitis) (Figure 2.3.4.7). Histological examination of the tail veins of the rest of the animals from group № 2 was not performed, but the uniformity of macroscopic changes suggests the presence of similar microscopic changes in these animals.

In group 7 (3500 mg/kg), in a single case, thickening of the caudal vein due to plethora of blood was found. One female from group 8 (dose 3750 mg/kg) was found to have a thickening of the vessel wall.

FINDINGS

The study of the toxic properties of test object Lithium Ascorbate, substance (Normopharm LLC, Russia) following a single intravenous administration to sexually mature rats suggested the following conclusions:

1. The picture of intoxication (inhibition of the general condition of various severity, ruffled hair) was manifested immediately after administration of the substance in doses of 1000 mg/kg and higher. The severity and frequency of signs of intoxication increased with increasing dose of the object. Mortality was predominantly delayed, with death occurring on days 3-5 after administration.

2. In routine necropsia, signs of local irritation (hemorrhagic impregnation of surrounding tissues, thrombus in the lumen of the artery) were found in the groups that received the test object in doses of 3000 mg/kg, 3500 mg/kg and 3250 mg/kg, 3500 mg/kg, 3750 mg/kg and 4000 mg/kg.

3. A dose of 3000 mg/kg is considered to be the maximum tolerated dose for males and 3500 mg/kg for females (the maximum doses studied in which no animal death was observed).

4. LD_{50} in males with intravenous administration - 3225±95.2 mg/kg, in females - 3625±62.5 mg/kg. According to the classification of toxicity of substances in parenteral methods of administration by Sidorov K.K., the tested object is assigned to the 6th class of low-toxic substances. According to the draft classification of chemicals proposed by Berezovskaya I.V., when administered intravenously, the object is assigned to the 4th class of low-toxic substances according to the draft classification GOST 12.1.007-76 (LD_{50} >700 mg/kg for intravenous administration to rats) and to hazard class 5 according to the draft GHS classification OECD (LD_{50} i/v > 700 mg/kg).

CONCLUSION

All study activity to investigate substance Lithium Ascorbate (Normopharm LLC, Russia) was planned and implemented in strict accordance with the requirements of the Ministry of Health of the Russian Federation and international standards in preclinical studies of the safety of pharmacological agents - the GLP system (Good Laboratory Practice) [20].

The pattern of intoxication was manifested immediately after administration of the substance in doses of 1000 mg/kg and higher. The severity and frequency of signs of intoxication increased with increasing dose of the object. LD50 in males when administered intravenously was 3225±95.2 mg/kg, in females 3625±62.5 mg/kg. According to the draft classification of chemicals proposed by Berezovskaya I.V., when administered intravenously, the object is assigned to the 4th class of low-toxic substances according to the draft classification GOST 12.1.007-76 (LD 50 >700 mg/kg when administered intravenously to rats) and to the 5th hazard class according to the draft classification of the GHS OECD (LD_{50} i/v > 700 mg/kg).

Signs of local irritant action, such as hemorrhagic impregnation of tissues at the injection site, thickening of the caudal vein, thrombus in the lumen of the artery were in doses of 3250 mg/kg, 3500 mg/kg, 3750 mg/kg, 4000 mg/kg, mainly in dead animals.

A dose of 3000 mg/kg can be considered as the maximum tolerated dose for males, and 3500 mg/kg for females, since no mortality of animals following intravenous administration of the substance in these doses has been observed.