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Research Article

The results of the study of the carcinogenic properties of glucosaminylmuramyldipeptide GMDP in chronic experiments in mice and rats

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Abstract

Introduction: The drug Licopid® (GMDP, glucosaminylmuramyldipeptide) is intended for complex therapy of conditions accompanied by secondary immunodeficiencies. This drug belongs to the group of microbial immunomodulators of bacterial origin. The transparent mechanism of action of the active substance GMDP allows putting the drug Licopid® into the category of promising immunotherapy drugs that are in demand in the complex therapy of many diseases resistant to traditional treatment. The objective of this study was to investigate the carcinogenicity of GMDP (Licopid®) in chronic animal experiments.

Materials and Methods: The study of the carcinogenic effects of GMDP was carried out in mice hybrids F1 CBAxC-57BL6 and Wistar rats of both sexes with intragastric administration five days a week (Mon-Tue-Wed-Thu-Fri) for 18 months at doses of 0.186 mg/kg, 1.86 mg/kg and 6.13 mg/kg to mice and for 21 months at doses of 0.086 mg/kg, 0.86 mg/kg and 2.83 mg/kg to rats.

Results and Discussion: The results of the clinical observation of the experimental animals in the course of the study demonstrated the absence of differences between mice and rats of the experimental and control groups.

Conclusion: Intragastric administration of GMDP to male and female mice for 18 months and rats for 21 months at the studied doses did not lead to tumor formation in the experimental animals.

Keywords

GMDP, glucosaminylmuramyldipeptide, Licopid®, carcinogenicity.

Introduction

The drug Licopid® is intended for complex therapy of the states which are followed by secondary immunodeficiency. This drug belongs to the group of microbial immunomodulators of bacterial origin. The active agent of the drug Licopid® – glucosaminylmuramyldipeptide GMDP. It is the ancestor of this group of immunomodulators and is an individual chemical compound, obtained by semi-synthetic means. In its structure, GMDP

corresponds to the minimal repeating biologically active fragment of a peptidoglycan molecule that makes up the cell wall of all gram-positive and gram-negative bacteria. GMDP and some of its structural derivatives, obtained in both semi-synthetic and synthetic ways, have found medical application due to the discovery of their adjuvant, immunomodulatory, detoxifying and anti-tumor properties, combined with pharmacological safety. In addition to GMDP, registered in the EEU countries under the trademark Licopid®, the following GMDP's structural derivatives are registered: in Japan – Romurtid (trade name Nopia®); in the EU – Mifamurtid (trade name Mepact®); in the Ukraine – Liasten®. Preparations are under clinical study: in the EU – MTP-PE, DTP-GDP (ImmTher) and in Russia – GMDP-A (glucosaminylmuramyldipeptide acid).

The specificity of GMDP and its derivatives is their ability to interact with the intracellular cytoplasmic receptors of the innate immunity of the NOD family expressed in phagocytic cells and to activate the innate immunity in the same way as the bacteria themselves (Girardin et al. 2003a, 2003b; Inohara et al. 2003).

It was proven that for GMDP (Licopid®) the interaction with the NOD2 receptor was absolutely specific and mediated the cellular and humoral adaptive immunity (Nesmeyanov 1998). A causal relationship was established between the ability to bind to PRR-pattern-recognition receptors of the NOD2 family and the ability to induce a cascade of processes responsible for the activation of the transcription factor NF-kB and the subsequent stimulation of immunity (Meshcheryakova et al. 2007). The belonging of GMDP to NOD2 agonists expressed in phagocytic cells reveals in detail the mechanism of stimulating the functions of immunocompetent cells. Such transparency of the GMDP mechanism of action allows attributing the drug Licopid® to the category of promising immunotherapy drugs that are in demand in the complex therapy of many diseases resistant to traditional treatment.

Earlier, it was proved there were no gene mutations and chromosomal aberrations when exposed to GMDP. However, the affinity of the GMDP molecule for NOD2 receptor molecules present not only in phagocytic cells, but also in other human cells and tissues, including some tumor myeloid lines (EMBL, EBI, ESTDAB, LSBM databases), served as the basis for a more detailed study of carcinogenic GMDP properties.

The objective of this study was to study the carcinogenicity of GMDP (Licopid®) in chronic animal experiments.

Materials and research methods

This study was conducted on the basis of the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University to the order of Peptek JSC.

The conduct of this study was approved by the local ethical committee for working with laboratory animals.

The study of the carcinogenic effects of GMDP was carried out in mice hybrids F1 CBAxC57BL6 and Wistar rats of both sexes with intragastric administration five days a week (Mon-Tue-Wed-Thu-Fri) for 18 months at doses of 0.186 mg/kg, 1.86 mg/kg and 6.13 mg/kg to mice and for 21 months at doses of 0.086 mg/kg, 0.86 mg/kg and 2.83 mg/kg to rats (it had been originally planned to administer the drug to rats for 24 months, however, taking into account the dynamics of mortality, it was decided to discontinue the study in rats). The above doses were calculated using interspecies conversion of daily doses for humans of 1 mg and 10 mg, as well as a three-time maximum daily dose of 10 mg – 33 mg, which equaled after conversion the maximum permissible dose for mice and rats, according to the coefficients in (Freireich et al. 1966). As the control, the data were used obtained from the intact animals (intragastric administration of an appropriate volume of solvent). For research, healthy females and male mice and rats were used, 50 animals of each sex in the group. The distribution within the groups was in accordance with body weight, with variation not exceeding 10%.

The animals were kept in standard conditions, in accordance with the approved Principles of good laboratory practice. The cages were individually ventilated Tecniplast systems for small laboratory animals. Feed was rodent pellet diet. Access to the feed was free. Water was purified, sterilized by UV irradiation. Access to water was free, through Tecniplast drinkers. The environmental conditions were the following: air temperature – within 20-26°C, relative humidity of air – within 30-70%; an artificial 12 h/12 h day/night cycle. Acclimatization and selection of animals for the study – quarantine for at least 10 days. Enrichment of the environment – Tecniplast mice houses.

The animals were monitored daily, recording the following information: their general condition, toxic events, deaths of animals, the occurrence of tumors. The animalas were weighed: during the first two months – weekly, during the 3rd and 4th months – twice a month, then – once a month. In the event of the death of the animal, complete necropsy and macroscopic examination of the internal organs of the animal weer performed as soon as possible for the presence of a tumor. A routine necropsy was performed on the bodies of mice and rats that had received the test drug for 18 and 21 months, respectively, and the organs and tissues were collected, followed by histological examination. The euthanasia method was placement in a CO_2 chamber.

The quantitative results were subjected to statistical processing by calculating the arithmetic mean (M), and standard error (±m). The intergroup differences were analyzed by parametric (Student's t-test) methods. The differences were considered statistically significant with $p<0.05$. For statistical calculations, Microsoft Excel and Statistica 10.0 were used.

Results and Discussion

The results of clinical observation of the experimental animals during the course of the study demonstrated the absence of differences between mice and rats of the experimental and control groups.

Thus, the general condition of the animals during the whole period of observation changed in accordance with age characteristics. No behavioral patterns were observed in the experimental animals different from those in the control animals.

The intensity and nature of motor activity during the entire observation period did not differ from those of the control animals and were characterized by gradual inhibition in accordance with age characteristics. No convulsions were observed in any animals. Neither the coordination of movements nor the skeletal muscle tone in the animals in the experimental groups were disturbed during the experiment, and did not differ from the corresponding indicators of the control animals.

The reaction to tactile, pain, sound and light stimuli was adequate in all the experimental animals; there were no differences between the experimental and control animals. The frequency and depth of respiratory movements in the experimental animals were visually indistinguishable from such in the animals from the control groups.

When observing the condition of the hair, seasonal moulting of the animals was noted. The condition of the skin of experimental animals was not different from that in the control animals.

No failures of the sense organs were registered in any animal after the start of injecting the test substance. During the administration of GMDP, the visible mucous membranes of the experimental animals, as well as of the control animals, remained pale pink in color; their humidity did not change; there were no symptoms of bronchitis, conjunctivitis and rhinitis.

The frequency of defecation and urination, the consistency of fecal masses and urine color of the experimental and the control animals did not visually change after the administration of GMDP.

No visual deviations in feed and water intake by the animals were observed.

During the course of the study, animal mortality associated with natural age-related changes was observed. Mortality was observed in the males to a higher degree than in the females (Figs 1-8).

Taking into account the dynamics of the lethality of the rats, it was decided to discontinue the study, to conduct necropsy followed by a histological examination of the organs and tissues of the rats that survived the intragastric administration of GMDP within 21 months.

Thus, by the time the study was completed, depending on the dose received, from 96% to 54% of male and from 96% to 78% of female mice, and from 0% to 4% of male and from 0% to 12% of female rats survived. No tumor formation was observed (Tables 1, 2).

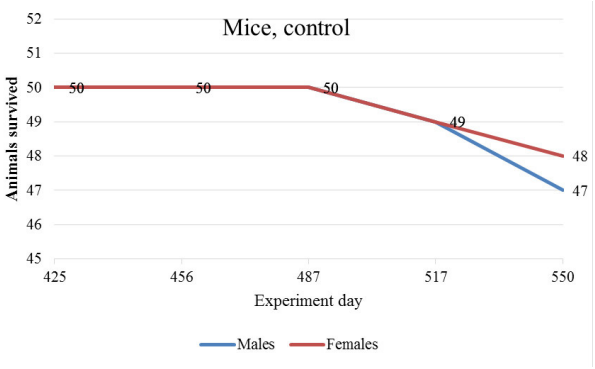

Figure 1. The dynamics of lethality in the group of control mice

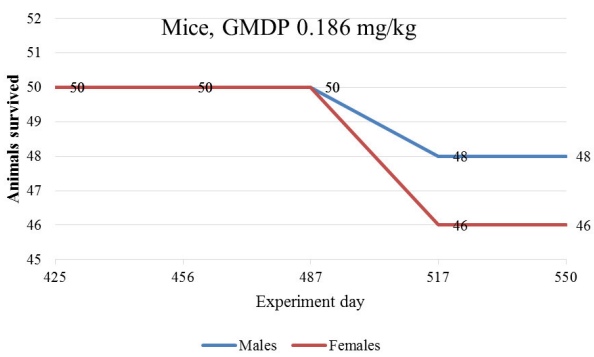

Figure 2. The dynamics of lethality in the group of mice treated with GMDP at a dose of 0.186 mg/kg

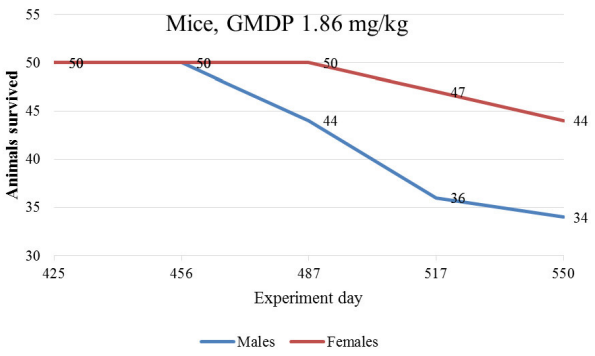

Figure 3. The dynamics of lethality in the group of mice treated with GMDP at a dose of 1.86 mg/kg

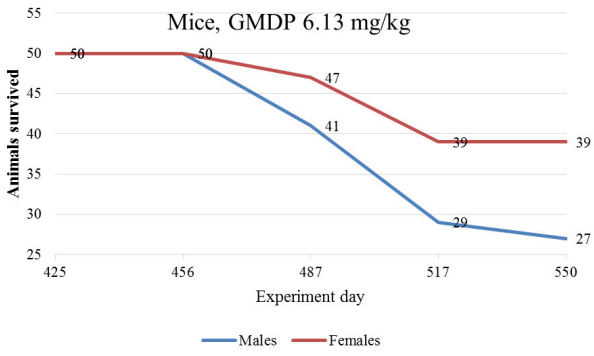

Figure 4. The dynamics of lethality in the group of mice treated with GMDP at a dose of 6.13 mg/kg

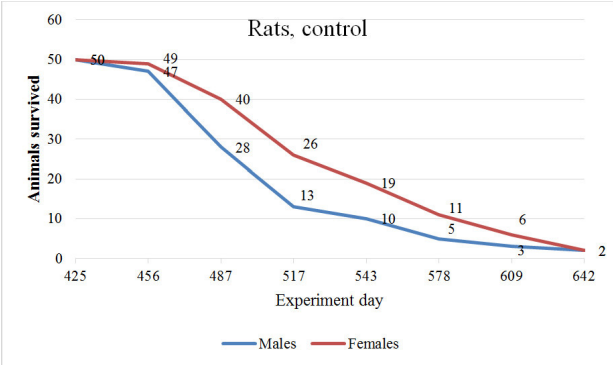

Figure 5. The dynamics of lethality in the group of control rats

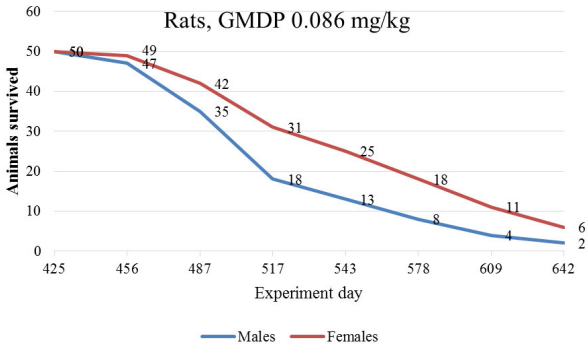

Figure 6. The dynamics of lethality in the group of rats treated with GMDP at a dose of 0.086 mg/kg

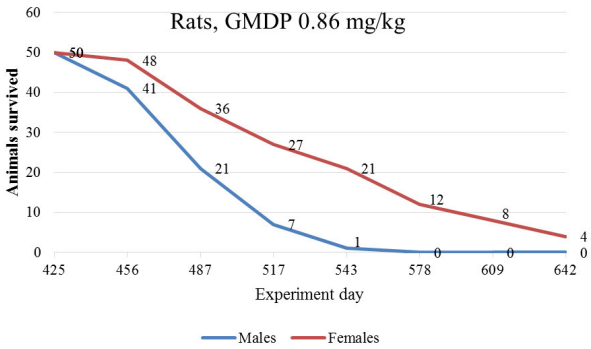

Figure 7. The dynamics of lethality in the group of rats treated with GMDP at a dose of 0.86 mg/kg

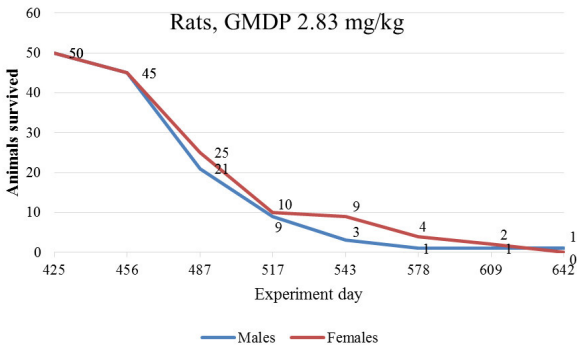

Figure 8. The dynamics of lethality in the group of rats treated with GMDP at a dose of 2.83 mg/kg

Table 1. The Number of Mice Which Survived by the End of the Study

Dose	Males		Females	
	Number of live animals	Timing of detecting tumors	Number of live animals	Timing of detecting tumors
Control	47 (94%)	Not found	48 (96%)	Not found
0.186 mg/kg	48 (96%)	Not found	46 (92%)	Not found
1.86 mg/kg	34 (68%)	Not found	44 (88%)	Not found
6.13 mg/kg	27 (54%)	Not found	39 (78%)	Not found

Table 2. The Number of Rats Which Survived by the End of the Study

Dose	Males		Females	
	Number of live animals	Timing of detecting tumors	Number of live animals	Timing of detecting tumors
Control	2 (4%)	Not found	2 (4%)	Not found
0.086 mg/kg	2 (4%)	Not found	6 (12%)	Not found
0.86 mg/kg	0 (0%)	Not found	4 (8%)	Not found
2.83 mg/kg	1 (2%)	Not found	0 (0%)	Not found

The body mass indicators of mice and rats, observed with the administration of GMDP at the doses studied, are given in Tables 3 and 4, respectively.

In the majority of the experimental animals during the experiment, up to the 427th day of observation in mice and up to the 489th day of observation in rats, there were no statistically significant differences in body weight indicators from those in the control groups; there were single cases of significant differences in body mass indicators in the control groups, both in terms of decreasing and increasing, without any signs of regularity. These observations can be explained by the individual characteristics of the animals.

Table 3. The Results of the Study of the Body Weight of Mice During the Study of the Carcinogenic Properties of GMDP

Group		Observation Day						
		1	**8**	**15**	**22**	**29**	**36**	**43**
Males control	M	17.08	18	19.42	20.66	21.66	22.46	23.54
	m	0.12	0.11	0.17	0.16	0.15	0.15	0.16
Males 0.186 mg/kg	M	16.86	18.06	19.62	20.44	21.2	22.76	23.4
	m	0.13	0.12	0.16	0.17	0.15	0.17	0.15
	p	0.208	0.709	0.398	0.345	0.033	0.192	0.526
Males 1.86 mg/kg	M	17.12	17.96	19.6	20.56	21.58	22.44	23.6
	m	0.11	0.11	0.15	0.16	0.16	0.15	0.15
	p	0.803	0.795	0.422	0.657	0.718	0.925	0.784
Males 6.13 mg/kg	M	17.1	18.04	19.72	20.68	21.46	22.2	23.62
	m	0.12	0.11	0.15	0.17	0.15	0.15	0.15
	p	0.905	0.799	0.189	0.932	0.355	0.232	0.719
Females control	M	17	18.18	19.12	20.52	21.36	22.52	23.52
	m	0.11	0.12	0.15	0.15	0.15	0.15	0.15
Females 0.186 mg/kg	M	16.8	17.96	19.66	20.62	21.58	22.5	23.88
	m	0.11	0.12	0.16	0.17	0.16	0.14	0.15
	p	0.219	0.194	0.015	0.668	0.314	0.923	0.092
Females 1.86 mg/kg	M	17.02	17.74	19.58	20.6	21.62	22.38	23.32
	m	0.12	0.12	0.16	0.16	0.16	0.17	0.16
	p	0.903	0.01	0.04	0.721	0.23	0.532	0.363
Females 6.13 mg/kg	M	17.04	18.16	19.52	20.22	21.84	22.32	23.46
	m	0.12	0.12	0.15	0.16	0.15	0.16	0.17
	p	0.808	0.905	0.064	0.184	0.027	0.359	0.789
		50	**57**	**64**	**78**	**92**	**108**	**124**
Males control	M	24.64	25.52	26.32	29.92	32.14	34.98	37.76
	m	0.15	0.16	0.15	0.2	0.26	0.26	0.26
Males 0.186 mg/kg	M	24.3	25.36	26.52	29.84	32.4	34.32	37.3
	m	0.17	0.17	0.15	0.23	0.26	0.26	0.24
	p	0.134	0.495	0.351	0.795	0.478	0.074	0.193
Males 1.86 mg/kg	M	24.48	25.38	26.66	29.8	32.18	34.5	37.64
	m	0.16	0.16	0.16	0.21	0.24	0.23	0.26
	p	0.46	0.542	0.128	0.68	0.91	0.167	0.742
Males 6.13 mg/kg	M	24.4	25.52	26.92	29.88	32.3	34.7	37.72
	m	0.16	0.15	0.16	0.21	0.24	0.24	0.26
	p	0.272	1	0.007	0.891	0.655	0.428	0.913
Females control	M	24.56	25.74	26.14	30	32.7	34.22	37.4
	m	0.17	0.16	0.14	0.23	0.27	0.25	0.27
Females 0.186 mg/kg	M	24.38	25.58	26.44	30.02	32.48	34.32	37.94
	m	0.17	0.16	0.17	0.22	0.24	0.23	0.24
	p	0.447	0.485	0.179	0.949	0.542	0.772	0.141
Females 1.86 mg/kg	M	24.4	25.76	26.52	29.84	32.62	34.8	37.66
	m	0.16	0.17	0.15	0.2	0.23	0.25	0.24
	p	0.496	0.931	0.069	0.598	0.82	0.105	0.474
Females 6.13 mg/kg	M	24.36	25.36	26.72	29.84	32.68	34.64	37.94
	m	0.15	0.17	0.17	0.22	0.23	0.25	0.24
	p	0.38	0.111	0.011	0.614	0.955	0.238	0.135

Table 3. Continued

Group		Observation Day						
		154	**185**	**215**	**246**	**277**	**305**	**336**
Males control	M	39.84	36.92	33.98	31.12	31.48	31.18	32.2
	m	0.25	0.3	0.35	0.38	0.33	0.31	0.31
Males 0.186 mg/kg	M	39.5	36.74	33.7	30.84	30.92	31.28	31.3
	m	0.29	0.3	0.4	0.37	0.33	0.37	0.38
	p	0.378	0.672	0.599	0.599	0.238	0.836	0.069
Males 1.86 mg/kg	M	39.5	37.5	33.7	29.98	30.78	31.18	31.5
	m	0.25	0.32	0.35	0.33	0.37	0.39	0.36
	p	0.341	0.185	0.574	0.027	0.165	1	0.142
Males 6.13 mg/kg	M	39.36	37.06	33.58	29.28	31.54	31.26	31.3
	m	0.25	0.33	0.37	0.32	0.34	0.35	0.37
	p	0.179	0.756	0.432	0	0.9	0.863	0.065
Females control	M	39.56	34.72	31.02	28.2	26.22	25.72	26.52
	m	0.24	0.35	0.33	0.41	0.5	0.5	0.55
Females 0.186 mg/kg	M	39.82	34	30.6	27.54	26.48	25.88	26.44
	m	0.23	0.31	0.38	0.42	0.53	0.55	0.49
	p	0.433	0.126	0.402	0.265	0.722	0.83	0.913
Females 1.86 mg/kg	M	39.98	34.34	30.88	27.56	26.7	26.24	26.9
	m	0.24	0.33	0.36	0.42	0.52	0.51	0.52
	p	0.218	0.426	0.773	0.281	0.504	0.468	0.617
Females 6.13 mg/kg	M	39.38	34.54	31.84	27.24	26.26	25.98	26.56
	m	0.22	0.34	0.31	0.37	0.58	0.55	0.55
	p	0.579	0.711	0.073	0.088	0.958	0.727	0.959
		366	397	427	458	489	519	550
Males control	M	32.44	32.32	32.1	32.2	32.04	31.69	32.17
	m	0.34	0.36	0.34	0.45	0.41	0.31	0.46
Males 0.186 mg/kg	M	31.88	31.5	31.9	32.28	32.38	32.69	31.67
	m	0.38	0.34	0.35	0.55	0.46	0.4	0.67
	p	0.277	0.1	0.684	0.911	0.582	0.051	0.537
Males 1.86 mg/kg	M	31.96	32.32	31.86	29.48	26.2	25.5	24.74
	m	0.37	0.38	0.38	0.47	0.61	0.51	0.98
	p	0.344	1	0.638	0	0	0	0
Males 6.13 mg/kg	M	31.88	32	31.78	29.82	26.63	24.79	23.67
	m	0.4	0.37	0.36	0.47	0.56	0.38	0.92
	p	0.294	0.533	0.518	0	0	0	0
Females control	M	26.78	26.56	25.76	26.96	28.46	28.82	28.92
	m	0.55	0.61	0.49	0.64	0.71	0.47	0.58
Females 0.186 mg/kg	M	26.82	25.4	25.98	28.48	28.96	29.98	29.02
	m	0.52	0.5	0.52	0.55	0.65	0.43	0.6
	p	0.958	0.146	0.76	0.075	0.604	0.07	0.9
Females 1.86 mg/kg	M	25.9	25.94	25.86	25.86	26.26	26.11	24.84
	m	0.55	0.53	0.56	0.51	0.48	0.41	0.41
	p	0.262	0.443	0.893	0.181	0.012	0	0
Females 6.13 mg/kg	M	26.42	25.98	26.62	25.84	23.91	22.97	25.13
	m	0.53	0.55	0.52	0.61	0.82	0.78	0.97
	p	0.64	0.481	0.232	0.207	0	0	0.001

Note: M – the arithmetic average; m – the standard error; p – the level of statistical significance in comparison with the control

Table 4. The Results of the Study of the Body Weight of Rats During the Study of the Carcinogenic Properties of GMDP

Group		Observation Day							
		1	**8**	**15**	**22**	**29**	**36**	**43**	**50**
Males control	M	174.68	176.3	177.38	179.8	182.48	185.46	187.56	190.28
	m	0.41	0.33	0.26	0.3	0.24	0.29	0.25	0.25
Males 0.086 mg/kg	M	174.92	176.56	177.78	180.12	182.22	185.1	187.26	189.78
	m	0.47	0.35	0.24	0.3	0.23	0.29	0.25	0.3
	p	0.703	0.589	0.259	0.451	0.433	0.388	0.395	0.206
Males 0.86 mg/kg	M	175.86	176.16	177.56	179.86	182.36	185	187.38	189.94
	m	0.45	0.38	0.23	0.28	0.26	0.31	0.25	0.3
	p	0.057	0.782	0.606	0.884	0.736	0.281	0.606	0.388
Males 2.83 mg/kg	M	175.3	176.18	177.58	179.92	183	185.7	187.38	187.62
	m	0.42	0.36	0.26	0.28	0.22	0.29	0.25	0.26
	p	0.294	0.805	0.587	0.77	0.113	0.559	0.608	0
Females control	M	164.5	165.72	167.68	170.12	172.42	175.26	177.48	180.26
	m	0.45	0.38	0.26	0.26	0.24	0.3	0.22	0.28
Females 0.086 mg/kg	M	165.3	165.88	167.22	170.08	172.5	174.88	177.38	180.2
	m	0.42	0.36	0.24	0.28	0.25	0.26	0.23	0.3
	p	0.201	0.762	0.199	0.917	0.815	0.343	0.756	0.884
Females 0.86 mg/kg	M	164.72	166.34	167.58	170.46	172.52	174.98	177.32	179.96
	m	0.45	0.33	0.24	0.27	0.24	0.3	0.25	0.26
	p	0.732	0.224	0.78	0.365	0.768	0.509	0.629	0.439
Females 2.83 mg/kg	M	165.32	165.7	167.28	170.18	172.14	174.48	177.62	180.34
	m	0.4	0.37	0.26	0.28	0.23	0.28	0.21	0.28
	p	0.176	0.97	0.278	0.875	0.399	0.063	0.645	0.84
		57	**64**	**78**	**92**	**108**	**124**	**154**	**185**
Males control	M	192.44	195.06	200.36	211.46	219.92	227.82	235.22	232.48
	m	0.27	0.26	0.43	0.33	0.45	0.6	0.46	0.76
Males 0.086 mg/kg	M	192.38	194.94	199.56	210.54	220.04	228.02	234.68	233.8
	m	0.23	0.28	0.45	0.37	0.47	0.63	0.45	0.73
	p	0.864	0.755	0.201	0.066	0.854	0.819	0.405	0.213
Males 0.86 mg/kg	M	192.42	195.02	200.3	210.74	219.96	227.74	234.64	233.34
	m	0.26	0.25	0.51	0.35	0.41	0.71	0.45	0.76
	p	0.957	0.912	0.928	0.142	0.948	0.931	0.371	0.428
Males 2.83 mg/kg	M	187.34	192.26	200.9	211.08	220.42	228.12	235.7	233.92
	m	0.25	0.22	0.42	0.32	0.46	0.7	0.48	0.84
	p	0	0	0.369	0.414	0.44	0.745	0.476	0.208
Females control	M	182.14	185.08	190.74	201.1	211.14	220.78	228.46	230.1
	m	0.24	0.27	0.4	0.49	0.49	0.52	0.33	0.41
Females 0.086 mg/kg	M	182.56	185.32	189.56	200.3	210.58	221.38	229.3	230.72
	m	0.23	0.25	0.44	0.53	0.48	0.51	0.37	0.38
	p	0.206	0.522	0.049	0.272	0.417	0.412	0.091	0.273
Females 0.86 mg/kg	M	182.44	185.28	190.08	201.66	211.4	220.5	228.96	230.7
	m	0.26	0.29	0.4	0.58	0.46	0.52	0.36	0.43
	p	0.39	0.62	0.248	0.464	0.7	0.705	0.308	0.319
Females 2.83 mg/kg	M	182.06	185.6	189.72	201.52	210.6	221.16	228.68	229.68
	m	0.24	0.3	0.42	0.54	0.44	0.51	0.35	0.4
	p	0.813	0.207	0.081	0.566	0.413	0.604	0.646	0.465

Table 4. Continued

Group		Observation Day							
		215	**246**	**277**	**305**	**336**	**366**	**397**	**427**
Males control	M	233.48	235.98	236.1	235.84	236.14	235.86	234.7	235.42
	m	0.63	0.62	0.55	0.64	0.65	0.64	0.59	0.7
Males 0.086 mg/kg	M	233.6	235.06	236.08	236.26	236.6	235.08	235.96	235.9
	m	0.67	0.66	0.56	0.57	0.54	0.69	0.71	0.63
	p	0.896	0.31	0.98	0.624	0.588	0.411	0.174	0.611
Males 0.86 mg/kg	M	233.88	234.7	235.44	235.18	235.64	235.22	235.52	234.76
	m	0.62	0.66	0.55	0.59	0.55	0.72	0.66	0.69
	p	0.651	0.158	0.395	0.449	0.561	0.51	0.356	0.503
Males 2.83 mg/kg	M	233.2	234.9	235.46	234.64	236.26	235.66	236	235.46
	m	0.63	0.68	0.55	0.57	0.53	0.71	0.6	0.67
	p	0.753	0.24	0.409	0.162	0.887	0.836	0.125	0.967
Females control	M	230.66	231.4	231.48	232.78	232.74	232.92	232.02	233.38
	m	0.52	0.67	0.68	0.64	0.67	0.66	0.62	0.68
Females 0.086 mg/kg	M	230.1	231.36	230.46	232.06	231.4	232.9	232.7	232.44
	m	0.54	0.67	0.52	0.71	0.66	0.69	0.63	0.62
	p	0.46	0.967	0.235	0.454	0.157	0.983	0.441	0.31
Females 0.86 mg/kg	M	231.56	232.52	232.66	231.28	232.34	231.18	232.98	232.84
	m	0.47	0.6	0.69	0.56	0.62	0.73	0.6	0.61
	p	0.206	0.216	0.224	0.081	0.662	0.08	0.268	0.556
Females 2.83 mg/kg	M	230.1	231.94	231.74	231.06	231.9	232.44	230.94	231.56
	m	0.54	0.69	0.72	0.63	0.58	0.66	0.63	0.66
	p	0.46	0.577	0.792	0.059	0.342	0.608	0.223	0.059
		458	**489**	**519**	**550**	**580**	**611**	**642**	
Males control	M	233.72	235	262.92	268.1	255.2	248.67	239	
	m	0.99	1.53	9.4	9.4	13.59	19.47	19	
Males 0.086 mg/kg	M	236.53	233.89	226.44	234	233.25	251	243	
	m	1.73	3.34	7.37	8.99	9.16	3.42	3	
	p	0.166	0.78	0.004	0.017	0.191	0.895	0.855	
Males 0.86 mg/kg	M	234.97	231.1	237.5	260	–	–	–	
	m	2.17	4.26	12.45	–	–	–	–	
	p	0.573	0.337	0.189	–	–	–	–	
Males 2.83 mg/kg	M	233.22	236.14	219.67	248	254	248	240	
	m	2.04	4.29	14.75	20	–	–	–	
	p	0.817	0.782	0.021	0.401	–	–	–	
Females control	M	234.17	233.5	230.46	216	215.09	209.67	221	
	m	1.51	3.26	6.02	7.26	7.7	10.06	7	
Females 0.086 mg/kg	M	234.31	234.4	227.8	207.68	212.89	223.09	211	
	m	1.39	2.31	4.25	7.87	8.42	9.06	6.49	
	p	0.946	0.82	0.714	0.455	0.86	0.366	0.447	
Females 0.86 mg/kg	M	236.6	236.14	224.62	216.67	228.17	228.5	229.5	
	m	1.77	2.66	5.27	5.02	4.63	3.72	3.1	
	p	0.298	0.539	0.468	0.941	0.153	0.074	0.25	
Females 2.83 mg/kg	M	235.74	238	220	220	242	243	–	
	m	2.03	2.7	7.86	11.12	6.98	11	–	
	p	0.526	0.343	0.36	0.774	0.07	0.131	–	

Note: M – the arithmetic average; m – the standard error; p – the level of statistical significance in comparison with the control

At the same time, in the subsequent observation period, in the mice treated with GMDP at doses of 1.86 mg/kg and 6.13 mg/kg, as well as in male rats treated with GMDP at doses of 0.086 mg/kg and 2.83 mg/kg, a statistically significant lag in body mass indexes from those in the corresponding control groups was observed, progressing towards the end of the study. At the same time, in the other groups of the experimental animals, in spite of the existing mortality, there were no statistically significant lags in body weight indexes from those in the control groups.

In accordance with the research plan, necropsy of the bodies of the dead animals and a macroscopic study of their internal organs for tumors were carried out. In addition, a routine necropsy was carried out on the bodies of all the surviving mice and rats that had received the test drug for 18 and 21 months, respectively, and the organs and tissues were recovered for subsequent histological examination.

No visual differences between the experimental groups of animals and the control animals were observed. Neither were any tumors detected. The overall macroscopic pattern was as follows.

Oral cavity. No pathological contents observed.

Tongue. Clean, regular shape, soft texture, pale color.

Esophagus. The mucosa of the esophagus was pale gray, smooth, shiny, with patent lumen.

Stomach. The stomach with well pronounced folds of the mucous membrane, the mucosa of pale gray color, moist, shiny, no pathological contents observed.

Pancreas. Pancreas in its typical location, pale gray in color, dense, fine granular in section.

Liver. The liver is dense, with a smooth surface, the lobes are pronounced, pale brown in section, anemic.

Large and small intestines. The contents of the intestines are adequate to each of their parts, the mucous membrane with definite folds, moist, pale gray in color, with patent lumen, no pathological contents observed.

Mucous membranes of larynx, trachea, main bronchi. Pale pink, smooth, moist, shiny, with patent lumen, no pathological contents observed.

Heart. The heart is rounded, well contracted. Under the epicardium – the usual venous pattern of the coronary vessels. The valve leaflets are thin, translucent. Endocardium is smooth and shiny.

Aorta. Elastic, smooth and clean intima, with patent lumen.

Kidneys. The kidney capsule strips easily; there is a smooth surface under it; in section – the kidneys have weak blood filling, with a clear lamellar structure; the mucous membranes of the pelvises and tje bladder are pale gray, moist, shiny, no pathological contents observed.

Adrenal glands. The adrenal glands are rounded, with the parenchyma clearly divided into the cortex and medulla, pale gray, not adhered to the surrounding tissues.

Spleen. The spleen in its typical location, with a smooth capsule, n section – of gray-red color; the splenic pulp does not scrape away.

Brain. The brain membranes are translucent, smooth, and shiny. The convolutions of the brain are well outlined. In section, the substance of the brain is wet, shiny, with a symmetrical pattern of the structure. The vessels of the base of the brain are arranged symmetrically, with collapsed walls.

Organs of the thoracic and abdominal cavities are located properly. The cavities are free from liquids and adhesions.

Lungs. The collapsed lungs, the lobes are distinguishable, pale gray in color, covered with thin pleura, with weak blood filling, no pathological contents observed.

Thymus. Thymus is bilobate, leaf-shaped, well identified among the organs of the anterior mediastinum. Tissue is elastic, homogeneous, pale gray.

Thyroid. Thyroid gland is reddish-pale, of normal size and shape, with moderately dense texture.

Testes and epididymises (in males). Testes and epididymises are of usual form, elastic consistency, and pale gray color. In secion, layers are visualized in the tesis tissue.

Ovaries (in females). Ovaries are gray-pink, with elastic consistency, surrounded with a small amount of adipose tissue. In section, follicles can be identified in the tissue**.**

Uterus (in females). Uterus septus, dense, with smooth surface; the color is pale yellowish-pink. The cavity is without pathological contents. The horns are thin, long, passable, with no pathological content observed.

The following organs and tissues of mice and rats that had received the test drug for 18 and 21 months were subjected to histological examination, respectively: stomach, small intestinal fragment, large intestinal fragment, liver, spleen, kidney, adrenal gland, bladder, brain, lung, heart, testis (in males), uterus and ovary (in females).

The study did not reveal any intergroup differences in the histological picture of the studied organs in the experimental animals in comparison with the control.

Conclusion

Thus, intragastric administration of GMDP (Licopid®) produced by Peptek JSC, Russia, to male and female mice for 18 months at doses of 0.186 mg/kg, 1.86 mg/kg and 6.13 mg/kg and to rats for 21 months at doses of 0.086 mg/kg, 0.86 mg/kg and 2.83 mg/kg does not lead to tumor formation in the experimental animals.

Given the fact that the excipients that make up Licopid® are traditional, well-studied additive agents that do not have carcinogenic activity, basing on the results obtained, it can be concluded that there are no carcinogenic properties in the finished pharmaceutical form of GMDP – Licopid®.

The obtained results expand knowledge about safety, in particular, carcinogenicity in long-term in-vivo experiments, of the active pharmaceutical substance GMDP and drug Licopid® manufactured by Peptek JSC, Russia.

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