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Lithium Chloride Effect on Mortality and Neurological Deficits in the Model of Ischemic Stroke in Rats

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ABSTRACTS The relevance of the problem of ischemic stroke is difficult to overvalue in modern terms. The data on the frequency of occurrence and outcomes, especially among young people, force us to look for new strategies to minimize its consequences. Recent experimental studies have shown pronounced neuro-cardio-nephroprotective properties of lithium salts.

AIM OF STUDY To evaluate the effect of lithium chloride on the lethality and severity of cognitive and neurological deficits in the modeling of ischemic stroke in rats.

MATERIAL AND METHODS The study used mongrel male rats weighing 312±12.5 g. The model of Longa's focal ischemia was used as a basis. The animals were divided into 5 groups: false-operated, control (model of ischemic stroke with the introduction of 0.9% NaCl) and three groups with the introduction of lithium chloride in various concentrations (4.2 mg/kg, 21 mg/kg and 63 mg/kg). The drug was administered daily for 14 days with a parallel assessment of neurological deficits.

RESULTS According to the results of the experiment, the following data were obtained with respect to lethality in the studied groups: false – operated 0 out of 8, control group – 13 out of 22 (lethality 59%), group 3 (LiCl 4.2 mg/kg) – 8 out of 14 (lethality 57%), p>0.05 with respect to control, group 4 (LiCl 21 mg/kg) – 6 out of 15 (lethality 40%) p>0.05 with respect to control and in group 5 (LiCl 63 mg/kg) – 4 out of 15 animals died (lethality 27%) p=0.0317. Lithium chloride at doses of 21 mg/kg and 63 mg/kg resulted in a decrease in the severity of neurological deficits on the second day of the experiment. On the 15th day of the experiment, there were no differences in the severity of neurological disorders. Also, the dosage of 63 mg/kg contributed to better memory retention during the assessment of cognitive functions.

CONCLUSION Lithium chloride at a dosage of 63 mg/kg significantly (p=0.037) reduced the mortality and severity of neurological deficits in the simulation of experimental ischemic stroke in rats compared to the control group.

Keywords: lithium, neuroprotection, ischemic stroke, neurological deficit, experimental study

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IS - ischemic stroke
HA - halogenated anesthetics
MCA - middle cerebral artery

INTRODUCTION

For quite a long time it was believed that the problem of cardiovascular diseases in general and strokes in particular is associated with life expectancy and the average age of the population of developed and developing countries. However, according to modern studies [1], it becomes clear that diseases such as heart attack and stroke have begun to occur in young people much more often. The percentage of deaths [2], as well as persistent disability of patients [3], who were treated according to the available protocols, is alarming. Unfortunately, far from all problems are solved by prevention and early diagnosis [4], and when it comes to the treatment of acute ischemic stroke (IS), the prognosis primarily depends on the timing of the start of treatment, which, due to various factors, may not always be optimal for a favorable prognosis [5]. In addition, such a procedure as thrombolysis (including selective), unfortunately, is not always available at various stages of hospitalization and / or is not performed in all hospitals, which further complicates the prognosis of this category of patients. These circumstances determine the search for drugs that can, if not completely prevent, then at least minimize the consequences and reduce mortality in such a formidable pathology.

Of the drugs with a neuroprotective effect, it is worth highlighting lithium salts separately. Tablets based on lithium carbonate have found application primarily in psychiatry and have been successfully used for the treatment of patients with bipolar disorders for more than 60 years [6, 7]. It is important to note that the risk of stroke in the group of patients with bipolar disorders treated with lithium preparations is significantly lower than in the general population [8, 9]. Recent studies by *S.E. Mohammadianinejad* [10] and *Y.R. Sun* [11] showed improvements in motor function in patients treated with lithium carbonate after a stroke. In addition, a relationship between lithium therapy and gray matter volume in humans has been proven, which potentially indicates the ability of the drug to reduce the risk of developing dementia [12, 13].

At the same time, in a number of experimental studies, both *in vivo* and *in vitro,* the neuroprotective properties of lithium salts were shown [14, 15]. Of particular significance is the work of *Ren Ming* [16], where the team of authors for the first time clearly demonstrated the neuroprotective effect of lithium on IS models in rats, which was expressed in a significant decrease in the volume of brain damage and neurological deficit. Similar conclusions

about the presence of neuroprotection were made by a number of other authors, including models of global cerebral ischemia [17] and hemorrhagic stroke [18]. In a recent experimental study by a group of authors, it was found that lithium chloride has a significant neuroprotective effect, which manifests itself in the preservation of the viability of neurons in the fields CA1 and CA3/CA4 of the hippocampus in the postresuscitation period after cardiac arrest. The authors note that the obtained results indicate the prospects for the possible use of lithium chloride in clinical practice for the correction of postischemic encephalopathies [19].

In this study, the effect of various concentrations of lithium chloride on such an important indicator of treatment effectiveness as mortality was studied for the first time.

The aim of the study was to evaluate the effect of lithium chloride on lethality and the severity of cognitive and neurological deficits in IS in rats.

Research objectives:

1. To evaluate the effect of lithium chloride at various concentrations on mortality in rats in the simulation of IS by 60-minute occlusion of the middle cerebral artery.

2. To evaluate the effect of lithium chloride at various concentrations on the severity of neurological and cognitive deficits in rats with IS.

3. To develop an effective strategy for reducing mortality and the severity of neurological and cognitive deficits by selecting the optimal dose of lithium chloride.

MATERIAL AND METHODS

Outbred male rats weighing 312 ± 12.5 g were used in the work. Long's model of focal ischemia was taken as a basis [20]. To exclude the influence of the researcher's preferences on the formation of experimental groups, the selection of animals was carried out using the method of modified block randomization [21]. To do this, all animals transferred to the study were randomly placed in the cells of the randomization block (the number of cells in the randomization block is a multiple of the number of groups in the experiment). Further, using a random number generator, a list of data was obtained containing the numbers of cells with animals and the corresponding numbers of groups where the animals were subsequently placed [22]. At each stage, each animal was marked by marking the base of the tail with a permanent marker. In accordance with the mark, each animal selected for the study was assigned an individual number.

The animals were divided into five groups. Group I (n = 8, average weight 300.2±12.5 g) was represented by sham-operated animals, in which a median skin incision was made along the projection line of the trachea as a surgical intervention and an artery was isolated, after which the wound was sutured in layers, and the suture treated with an antiseptic. Group II was the control group (n =22, mean weight 304±11.7 g), the animals were subjected to focal cerebral ischemia by occlusion of the middle cerebral artery (MCA). In this group, for 14 days, a solution of sodium chloride 0.9% was intravenously injected daily at a rate of 1.5 ml/kg. Group III (n = 14, mean weight 305±12.1 g) — after stroke modeling, the animals received intravenous lithium chloride once a day at a dose of 4.2 mg/kg. Group IV (n =15, mean weight 304±11.9 g) — after occlusion, the animals received intravenous lithium chloride at a dose of 21 mg/kg once a day. Group V (n =15, mean weight 306±10.2 g) — after focal ischemia, the animals received intravenous lithium chloride once a day at a dose of 63 mg/kg.

An increase in the number of animals in the control group was associated with a high mortality characteristic of the model and the need for additional studies to obtain statistically significant results.

In calculating the dosages of lithium chloride, we were guided by previously obtained data on toxicity (both chronic and acute) [7] (Fig. 1).

Fig. 1. Diagrammatic representation of the therapeutic range of lithium use, depending on the pathology, as well as toxic concentrations in blood plasma [7]

When modeling IS, general anesthesia was used with a combination of Zoletil 100 and Xylazine. Focal cerebral ischemia in rats was induced by MCA occlusion. To do this, the anesthetized animal was fixed in the supine position on a heated operating table (to exclude the mechanisms of protective hibernation). The neck area was shaved, treated with a skin antiseptic. Then a median skin incision was made along the projection line of the trachea. The muscles were separated by a blunt method, the common carotid artery and its branches, the external and internal carotid arteries, were isolated on the left side of the neck. A temporary ligature was applied to the common carotid artery. Distal to the bifurcation, two ligatures were applied to the external carotid artery, between which an incision was made. A filament (4-0 nylon thread) was inserted through a segment of the external carotid artery into the internal carotid artery, which was advanced to a distance of about 24 mm from the bifurcation of the common carotid artery to the beginning of the MCA branch. Thus, the base of the MCA was blocked. Then the filament was fixed to the external carotid artery, the section of the external carotid artery was completely ligated. The blood supply was restored in the common carotid artery by removing the ligature from it. After that, the postoperative wound was sutured layer-by-layer, the suture was treated with an antiseptic. A schematic representation of the focal ischemia model is shown in Fig. 2.

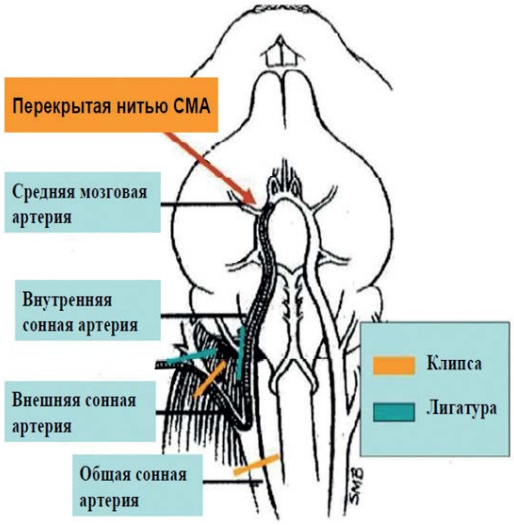

Fig. 2. Modeling of focal cerebral ischemia in rats by Longa's method

Note: MCA - middle cerebral artery

In sham operated anesthetized animals (group I), a median skin incision was made along the projection line of the trachea, and an artery was isolated. After that, the postoperative wound was sutured layer-by-layer, the suture was treated with an antiseptic.

The introduction of lithium chloride in groups III–V and sodium chloride in group II was carried out intravenously 15 minutes after fixation of the filament. The administration of the test preparation and control substance was carried out once daily for 14 days. Every day, the animals were examined in cages in order to detect lethality and pronounced abnormalities. To assess the neurological status of animals, a number of tests were used to characterize the preservation of the main reflexes [23, 24] (Table 1).

Table 1

Scale for assessing neurological status in animals

No.	Indicator	Points
one	Ataxia	
	No Yes	0 1
2	Impaired coordination	
	No Yes	0 1
3	stereotypical movements	
	No Weakly expressed Strongly expressed	0 1 2
4	Response to acoustic stimulus	
	Yes No	0 1
five	Palpebral closure	
	Eyelids open wide Ptosis is mild Ptosis is pronounced	0 one 2
6	Lateral reflex	
	Saved Missing	0 1
7	Grasp reflex	
	Two paws One paw Missing	0 1 2
8	Corneal reflex (corneal reflex)	
	Saved Missing on one side Missing on both sides	0 1 2
nine	Flexor reflex	
	No Missing on one leg Absent on both legs	0 1 2
10	Negative geotaxis	
	Yes No	0 1

On the 15th day, all surviving animals were subjected to humane euthanasia using carbon dioxide (CO_2) and subsequent exsanguination from the heart cavities. This was in line 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 September 22, 2010 [25].

The cognitive status of the animals was assessed using the Morris Water Maze test [26]. The training was carried out on the 7th–10th days of the experiment, the test was carried out on the 14th day after the formation of the pathology.

Statistica 10.0 (*StatSoft*, *Inc.*) and *MedCalc* 12.5.0.0 (*MedCalc Software bvba*) were used for statistical analysis. Mean values were represented by the median with an interquartile interval. Intergroup differences in indicators were assessed using the Mann–Whitney *U* -test and were considered statistically significant at $p < 0.05$.

RESEARCH RESULTS

With regard to mortality by groups, we obtained the data presented in Table 2. Statistically significant when calculating using Fisher's exact test, mortality differed from the control group only in group V ($p = 0.027$). There was no statistically significant difference in groups III ($p > 0.05$) and IV ($p > 0.05$).

Table 2

Total mortality after the experiment

Stroke simulation	Preparations	Dose, ml/kg	Quantity of males (deaths/total)	Mortality, %
No	Saline	1.5	0/8	0
Yes		1.5	13/22	59
	lithium chloride	4.2	8/14	57
		21	6/15	40
		63	4/15	27

When assessing daily mortality, it was noted that the largest number of deaths occurred on the 2nd–3rd day after stroke modeling. This table did not take into account the group of sham-operated animals, since mortality was zero in it (Table 3). On day 1, when assessing the proportion of dead animals from the total number, mortality in the control group was statistically significantly higher than mortality in groups III, IV and V (using Student's t-test, $p < 0.05$). Thereafter, no significant difference was noted. The most common cause of lethality on days 1–5 in the control group (9 animals (81.81%) out of 11) was acute cerebral insufficiency against the background of progressive cerebral edema. In 2 animals (18.19%) out of 11, death occurred as a result of progressive cardiovascular insufficiency, which, however, was also associated with post-stroke changes in the brain. Further lethality (15.38% of all deaths) was associated with secondary complications after stroke (hypoxia due to respiratory failure and hypoventilation pneumonia).

In group III, in 100% of cases, the cause of death was progressive cerebral edema due to stroke. Death on the 9th, 10th and 11th days (37.5% of all deaths) occurred as a result of secondary complications. In group IV, from days 1 to 5, 4 out of 5 animals (80%) died as a result of acute cerebral insufficiency against the background of cerebral edema, and 1 (20%) died from intracerebral hemorrhage. Further lethality (16.66% of the total number of deaths) was associated with developing pneumonia. In group V, 100% of the animals that died from days 1 to 5 had acute IS as the cause of death. Death on the 6th and 10th days after surgery (50% of the total number of deaths) occurred as a result of developed pneumonia.

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Table 3

Distribution of mortality by days of experiment (number/percent)

Stroke simulation	Preparations	Dose, ml/kg	Research Day												
			2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
Yes	Physiological solution, *n* (%)	1.5	5 (22.7)	2 (9.09)	–	4 (18.18)	–	1 (4.54)	–	–	–	–	–	–	1 (4.54)
	Lithium chloride, *n* (%)	4.2	1 (7.14) [a]	3 (21.42) [a]	1 (7.14) [b]	–	–	–	–	1 (7.14) [b]	1 (7.14) [b]	1 (7.14) [b]	–	–	–
		21	2 (13.33) [a]	1 (6.66) *b*	1 (6.66) [b]	1 (6.66) [a]	–	–	1 (6.66) [b]	–	–	–	–	–	–
		63	1 (6.66) [a]	1 (6.66) [b]	–	–	1 (6.66) [b]	–	–	–	1 (6.66) [b]	–	–	–	–

Notes: [a] — statistically significant (p<0.05) decrease in mortality in relation to the control group; [b] — statistically insignificant (p>0.05) change in mortality in relation to the control group

In the distribution of the structure of mortality by nosology in the control group, the cause of 9 deaths (69.23%) out of 13 was the stroke itself, the remaining 4 cases (30.77%) were associated with secondary complications. In group III, stroke was identified as the main cause of death in 5 animals (62.5%) out of 8, 3 (62.5%) out of 8 died from secondary complications. In group IV, death from stroke was recorded in 4 out of 6 animals (66.66%), 2 (33.34%) of 6 died from secondary complications. In group V, 2 out of 4 animals died as a result of stroke (50%), and another 2 (50%) out of 4 as a result of secondary complications. Table 4 shows the cumulative mortality rate in laboratory animals.

Table 4

Cumulative mortality rate by day of experiment

Stroke simulation	Preparations	Dose, mg/kg	Research Day												
			2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
Yes	Sodium chloride, *n* (%)	1.5	5 (22.7)	7 (31.79)	7 (31.79)	11 (49.97)	11 (49.97)	12 (54.51)	12 (54.51)	12 (54.51)	12 (54.51)	12 (54.51)	12 (54.51)	12 (54.51)	13 (59.05)
	Lithium chloride, *n* (%)	4.2	1 (7.14) [a]	4 (28.56) [b]	5 (35.7) [b]	5 (35.7) [b]	5 (35.7) [b]	5 (35.7) [a]	5 (35.7) [a]	6 (42.84) [b]	7 (49.98) [b]	8 (57.12) [b]	8 (57.12) [b]	8 (57.12) [b]	8 (57.12) [b]
		21	2 (13.33) [a]	3 (19.99) [b]	4 (26.65) [b]	5 (33.31) [b]	5 (33.31) [b]	5 (33.31) [a]	6 (39.07) [b]	6 (39.07) [b]	6 (39.07) [b]	6 (39.07) [b]	6 (39.07) [b]	6 (39.07) [b]	6 (39.07) [b]
		63	1 (6.66) [a]	2 (13.32) [a]	2 (13.32) [a]	2 (13.32) [a]	3 (19.98) [a]	3 (19.98) [a]	3 (19.98) [a]	3 (19.98) [a]	4 (26.64) [a]	4 (26.64) [a]	4 (26.64) [a]	4 (26.64) [a]	4 (26.64) [a]

Notes: [a] — statistically significant (p<0.05) decrease in mortality in relation to the control group; [b] — statistically insignificant (p>0.05) change in mortality in relation to the control group

When assessing cumulative mortality (Table 4), a statistically significant (p <0.05) decrease in mortality was obtained in groups III, IV and V on the 2nd day after a stroke in relation to the control. By the 3rd day, the decrease in mortality was statistically significant only in group V. Group III showed a statistically significant decrease in cumulative mortality on the 7th and 8th days (p <0.05), but later on the difference became statistically insignificant (p > 0.05). In group IV, cumulative mortality also significantly decreased by the 7th day after a stroke (p <0.05), but later on the difference lost its statistical significance (p >0.05). In group V, a statistically significant decrease in cumulative mortality was noted throughout the experiment (p <0.05).

Lithium chloride at doses of 21 and 63 mg/kg led to a decrease in the severity of the neurological deficit on the 2nd day of the experiment (Table 5), however, when assessing the neurological deficit on the 15th day, we did not obtain a statistically significant difference (p >0, 05) between the control group and groups III–V (the Kruskal–Wallis test was used followed by intergroup comparison of mean ranks).

Table 5

Neurological assessment status results, score, Me (Q1; Q3)

Stroke simulation	Preparations	Dose, ml/kg	2nd day of experiment	15th day of experiment
No	Saline	1.5	0.0 (0.0; 0.0) *n* =8	0.0 (0.0; 0.0) *n* =8
Yes		1.5	3.0 (2.0; 5.0) *n* =17	0.0 (0.0; 1.0) *n* =9
	lithium chloride	4.2	5.0 (3.0; 6.0) *n* =13	0.5 (0.0; 1.0) *n* =6
		21	2.0 (0.0; 4.0) *n* =13	0.0 (0.0; 1.0) *n* =9
		63	2.0 (1.0; 3.0) *n* =14	0.0 (0.0; 1.0) *n* =11

When assessing cognitive impairment, we took into account two indicators - the effect of lithium chloride on the process of learning to pass the Morris water maze (Table 6) and on learning outcomes (Table 7). If, in the case of learning speed, the data in groups III–V did not statistically significantly differ from the control (Tukey's test, p >0.05 was used in the analysis), then when passing the Maurice water maze, group V showed results that were almost identical to the group of sham-operated animals, statistically significantly different from the control group (using Student's t-test, p <0.05). The results in groups III and IV did not differ statistically significantly from the control group (p >0.05).

Table 6

Influence of studied drugs on the learning process, Morris Water Labyrinth test, sec, M±SEM

Stroke simulation	Preparations	Dose, ml/kg	*n*	Latent period of the platform finding, s	Total time spent in the sector, s	Number of sector visits
No	saline solution	1.5	8	8±1.4	24±2.8	4±0.7
Yes		1.5	9	17±2.6	15±1.3	2±0.3
	lithium chloride	4.2	6	14±6.2	16±2.3	3±0.8
		21	9	15±3.8	14±2.8	3±0.4
		63	11	8±1.5	19±2.1	4±0.6

Table 7

Learning outcomes, Morris Water Labyrinth test, M±SEM

Stroke simulation	Preparations	Dose, ml/kg	*n*	Latent period of the platform, s	Total time spent in the sector, s	Number of sector visits
No	Saline	1.5	8	8±1.4	24±2.8	4±0.7
Yes		1.5	9	17±2.6	15±1.3	2±0.3
	lithium chloride	4.2	6	14±6.2	16±2.3	3±0.8
		21	9	15±3.8	14±2.8	3±0.4
		63	11	8±1.5	19±2.1	4±0.6

DISCUSSION

Today, speaking about drugs with a proven neuroprotective effect in experimental studies, it is worth mentioning several groups of drugs. First of all, these are halogen-containing anesthetics (HA), for which the effect of anesthetic preconditioning has been described [27]. The neuroprotective effect of HA is realized in several ways. Firstly, it is a decrease in the metabolic activity of the brain, which increases the time of ischemic tolerance. Second, GA can regulate the content of intracellular calcium, the excess concentration of which plays an important role in ischemic brain damage [28, 29]. Finally, thirdly, HA through an increase in the concentration of reactive oxygen species in the cell [30] phosphorylate the enzyme glycogen synthase kinase 3beta (GSK-3 β) — a key link in limiting nonspecific mitochondrial permeability, thereby realizing the effect of anesthetic pre- and postconditioning [31].

Of great interest are drugs that have a direct inhibitory effect on the key enzyme in the HSC-3 β πρεχονδιτιονινγ μεχηανισμ [32], lithium salt [33]. In addition to the effect on HSC-3 β, the mechanisms of lithium neuroprotection are also implemented due to the inactivation of NMDA (N-methyl-D-aspartate) receptors [34], a decrease in the activity of the proapoptotic p53 protein and an increase in the activity of the antiapoptotic Bcl-2 proteins [35], as well as activation of the PI3K/Akt signaling pathway responsible for cell survival [36].

We should also mention xenon, whose effect on NMDA receptors [37] suggested that it has a neuroprotective effect, which was confirmed in *in vitro* studies on models of glutamate excitotoxicity and oxygen–glucose deprivation [38]. *In vivo studies* in models of IS and hemorrhagic stroke have also confirmed the neuroprotective effects of xenon inert gas [39-41].

With regard to the use of lithium chloride, there is some concern about its dose-dependent toxic effects [42], which need further study [7]. It is important to note that in recent experimental studies, lithium chloride demonstrated, in addition to neuroprotective properties, the effects of cardioprotection in a model of myocardial infarction in rats [43] and nephroprotection in a model of gentamicin nephrotoxicity [44]. Previously, the neuroprotective effect of lithium chloride was shown in a model of IS in rats, which was expressed in a significant decrease in the volume of brain damage [16]. The effect of lithium chloride on the volume of cerebral edema in IS was shown [45]. However, such an important indicator of the effectiveness of ongoing therapy as lethality in IS, as well as the effect on it of various concentrations of lithium chloride during long-term administration, was shown for the first time.

In the course of this study, answers were obtained to several fundamental questions. First, in the experiment, we made sure that this model of cerebral ischemia corresponds to the clinical picture, which was shown in the course of identifying neurological and cognitive impairments, as well as assessing mortality (taking into account the mortality of animals that did not receive specific therapy) [46]. Secondly, the effect of lithium chloride in various dosages on mortality and the severity of neurological and cognitive deficits in IS was revealed. And thirdly, a significant dose-dependent effect of its neuroprotective properties was shown. Undoubtedly, the discovery that the most effective concentration of lithium chloride in relation to neuroprotection was 63 mg/kg, which exceeds the optimal cardio- and nephroprotective doses (30 mg/kg) 2-fold, indicates the need for further careful study of this drug.

CONCLUSION

As a result of the study, data on the presence of a neuroprotective effect of lithium chloride in a model of ischemic stroke in rats were confirmed, which has a significant dose-dependent nature and for the first time showed a decrease in cognitive impairment and a statistically significant decrease in mortality with long-term administration.

1. Lithium chloride administered for 14 days to laboratory animals with ischemic stroke contributes to a statistically significant decrease in mortality at a dose of 63 mg/kg ($p = 0.027$).

2. Lithium chloride, administered for 14 days to laboratory animals with ischemic stroke at doses of 21 and 63 mg/kg, leads to a decrease in the severity of neurological deficit on the 2nd day of the experiment. With regard to cognitive impairment, a statistically significant improvement was demonstrated by the administration of lithium chloride at a concentration of 63 mg/kg compared with the control group ($p < 0.05$).

3. The greatest neuroprotective effect has the introduction of lithium chloride at a dose of 63 mg/kg, which is confirmed by a statistically significant decrease in mortality in relation to the control group by more than 2 times, as well as maintaining a level of cognitive status comparable to that in sham operated animals.

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