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Convergent Antidepressant-Like and Stress-Attenuating Effects of Lithium Ascorbate in Rodent Behavioral and Physiological Models

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Abstract

Background: Lithium salts differ in their chemical structure and may therefore exhibit distinct pharmacological and biological properties. Lithium ascorbate, an organic lithium salt, has been proposed to possess stress-attenuating and antidepressant-like effects, but its activity requires evaluation across complementary behavioral and physiological models.

Objective: To assess the behavioral and physiological effects of lithium ascorbate across three rodent studies and to compare its activity with lithium carbonate, ascorbic acid, and other organic lithium salts under the reported experimental conditions.

Methods: Three preclinical experiments were analyzed. In C57BL/6J mice, lithium ascorbate and comparator treatments were administered orally for 14 days, followed by assessment in the tail suspension test and a simulated transport-stress model. In outbred male rats, lithium carbonate, lithium succinate, lithium ascorbate, and lithium orotate were administered orally for seven days, and locomotor, exploratory, and anxiety-related behavior was evaluated in the open field test. In male Wistar rats, lithium-containing preparations were administered intraperitoneally for 14 days, and behavior was assessed in a modified forced swim test during treatment and after withdrawal.

Results: Lithium ascorbate consistently reduced immobility and increased active or sustained coping behavior. In the tail suspension test, immobility decreased by approximately 27% on day 7 and 31% on day 14 relative to controls, while active mobility increased by approximately 83% and 108%, respectively. Following simulated transport stress, lithium ascorbate reduced adrenaline by approximately 56–65% and norepinephrine by approximately 46–54%, while circulating eosinophil counts were higher than in controls. In the open field test, lithium ascorbate increased horizontal and vertical locomotor activity, hole exploration, and center entries, while reducing grooming and defecation. In the forced swim test, lithium ascorbate reduced immobility by approximately 33% on day 7 and 45% on day 14 and increased passive swimming. Part of this effect remained detectable after treatment withdrawal. Lithium carbonate generally produced weaker or negligible effects under the tested conditions.

Conclusion: Across complementary rodent models, lithium ascorbate demonstrated a consistent pattern of antidepressant-like, exploratory, and stress-attenuating activity. The convergence of behavioral and physiological findings supports further investigation of this compound.

Keywords: lithium ascorbate; organic lithium salts; lithium carbonate; antidepressant-like activity; stress response; anxiolytic-like activity; forced swim test; tail suspension test; open field test; simulated transport stress; catecholamines; exploratory behavior; rodent models; preclinical study; behavioral pharmacology.

Introduction

Stress-related disorders are characterized by interactions among behavioral, neuroendocrine, autonomic, and metabolic processes. Exposure to acute or repeated stress activates adaptive physiological systems that support short-term survival; however, excessive or prolonged activation may contribute to anxiety-related behavior, motivational impairment, anhedonia, cognitive dysfunction, and depressive symptoms (McEwen, 2000; Kyrou & Tsigos, 2009;

Herman et al., 2013). Experimental models that assess several dimensions of the stress response are therefore useful for the preclinical evaluation of compounds with potential stress-attenuating or antidepressant activity.

Lithium has a well-established role in psychiatric pharmacotherapy, particularly in the treatment and prevention of mood episodes in bipolar disorder (Fiorillo et al., 2023; Kessing, 2024). It also has clinically relevant anti-suicidal and augmentation effects in selected patients with depressive disorders (Cipriani et al., 2013). Nevertheless, the therapeutic use of conventional lithium preparations is limited by a relatively narrow therapeutic range, interindividual variability in pharmacokinetics, and the need for clinical monitoring (Gitlin, 2016; McKnight et al., 2012). These limitations have prompted continued investigation of lithium-containing compounds that may differ in bioavailability, tissue distribution, pharmacodynamic properties, and tolerability.

The biological properties of a lithium salt may depend not only on the lithium cation but also on the accompanying anion. Inorganic lithium salts, such as lithium carbonate, and organic lithium salts, including lithium ascorbate, lithium succinate, and lithium orotate, differ in molecular composition and may consequently differ in absorption, transport, metabolism, and biological activity. Organic anions may also exert biological effects independently of lithium. Accordingly, equal compound doses of different lithium salts cannot be assumed to produce equivalent systemic lithium exposure or identical pharmacological responses (Torshin et al., 2022).

Lithium ascorbate is an organic salt containing lithium and the ascorbate anion. Previous experimental work has suggested that this compound may have neuroprotective, stress-attenuating, anxiolytic-like, and antidepressant-like properties (Torshin et al., 2022). However, evidence based on a single behavioral paradigm is insufficient to characterize a compound's activity in complex stress-related states. Behavioral tests differ in their construct validity and sensitivity to locomotor activation, novelty, environmental stress, repeated exposure, and species-specific behavior (Commons et al., 2017; Prut & Belzung, 2003; Can et al., 2012a; Can et al., 2012b). A more informative preclinical assessment therefore requires convergence across independent models and outcome domains.

The forced swim and tail suspension tests are commonly used to evaluate antidepressant-like behavioral effects in rodents (Can et al., 2012a; Can et al., 2012b). In both paradigms, a reduction in immobility is conventionally interpreted as increased active coping, although immobility should not be regarded as a direct equivalent of human depression (Porsolt 1977; Porsolt 1978; Porsolt 1979; Porsolt 2000). Analysis of distinct behavioral components, including climbing, active mobility, and swimming, may provide additional information beyond total immobility time (Detke, 1995; Commons et al., 2017).

The open field test provides a complementary assessment of spontaneous locomotion, exploratory activity, avoidance of exposed areas, and autonomic or emotional responses to a novel environment (Prut & Belzung, 2003). Measures such as horizontal movement, rearing,

investigation of holes, entry into central areas, grooming, and defecation can be used to characterize the balance between exploration and defensive behavior. Interpretation requires caution because changes in these measures may reflect locomotor stimulation, sedation, altered motivation, anxiety-related behavior, or combinations of these processes (Seibenhener & Wooten, 2015).

Physiological models provide an additional level of assessment. Simulated transport stress produces combined vestibular, mechanical, social, and environmental stimulation and can activate the sympathoadrenal and hypothalamic–pituitary–adrenal systems (Kvetnansky et al., 2013; Kyrou & Tsigos, 2009). Post-stress catecholamine concentrations and leukocyte redistribution may therefore serve as indirect indicators of systemic stress activation, although these measures are sensitive to sampling conditions, circadian timing, handling, and assay procedures.

The present work integrates findings from three preclinical experiments evaluating lithium ascorbate in complementary rodent models. The first experiment assessed behavioral and physiological responses in mice using the tail suspension test and simulated transport stress. The second compared organic and inorganic lithium salts in rats using the open field test. The third examined the effects of repeated administration and subsequent withdrawal of lithium-containing preparations in a modified forced swim test.

The primary objective was to determine whether lithium ascorbate produced a consistent pattern of antidepressant-like and stress-attenuating activity across different experimental paradigms. A secondary objective was to compare the observed effects with those produced by ascorbic acid, lithium carbonate, and other organic lithium salts under the conditions of the individual experiments. It was hypothesized that lithium ascorbate would reduce passive or immobility-related behavior, support exploratory activity, and attenuate physiological indices of stress activation.

Lithium Ascorbate

One of the latest developments in terms of lithium salts on the pharmacological market is lithium ascorbate by Normopharm (Torshin et al., 2022). Lithium ascorbate is an organic salt of lithium and ascorbic acid (Vitamin C).

Lithium ascorbate is a lower-toxicity lithium salt with reported potential anxiolytic, antidepressant, neuroprotective, and memory-supporting effects (Torshin et al., 2022; Rastashanskiy, 2020; Rastashanskiy & Ostrenko, 2020).

Although lithium ascorbate has been proposed as a potentially lower-toxicity organic lithium salt with neuropsychiatric relevance, its use as a pharmacologically active compound requires systematic preclinical safety characterization, including repeated-dose toxicity, genotoxicity-

related endpoints, reproductive and immunotoxicity, allergenic potential, and effects on major physiological systems.

The tested drug developer and the organization responsible for arranging the preclinical studies is Normopharm LLC. The studies were performed in accordance with GLP OECD principles (OECD, 1998a).

The test substance was Lithium Ascorbate, substance code T-1.28/21, supplied by Normopharm LLC, Russia. The substance was manufactured according to Good Manufacturing Practice. The control substance was listed as vehicle code M-1.28/21; saline solution, Grotex LLC, Russia.

Study 1: Behavioral and Physiological Effects of Lithium Ascorbate During Tail Suspension and Simulated Transport Stress

The antidepressant-like and stress-protective effects of lithium salts and lithium-containing combinations were investigated in C57BL/6J mice using the tail suspension test and a model of simulated transport stress. Seventy-two mice were allocated to six groups of 12 animals matched by age, body weight, and sex. The test compounds were administered orally by gastric gavage once daily for 14 days in a volume of 2 mL per animal. Behavioral and physiological assessments were conducted on days 7 and 14 of treatment.

Group	Dosage (mg/kg)	
1	Ascorbic acid	10.00
2	Lithium ascorbate	10.00
3	Lithium carbonate + Ascorbic acid	1.70
	Lithium carbonate	10.00
4	Lithium carbonate + Ascorbic acid	50.00
5	Control	50.00
	Lithium ascorbate	10.00
6	Lithium carbonate + Ascorbic acid	-

Table 1. Dosage calculation by groups

The reported treatments included ascorbic acid, lithium ascorbate, lithium carbonate, and combinations of lithium carbonate with ascorbic acid (Table 1).

For the tail suspension test, each mouse was suspended by the tail for six minutes, and the complete session was video-recorded. A trained observer who was reported to be blinded to treatment allocation

quantified active mobility, passive mobility, and immobility. Mobility included hind-limb movements and other escape-oriented behaviors. To prevent tail climbing, which is common in C57BL/6J mice, a Plexiglas cylinder was placed around the tail during testing (Can et al., 2012).

Lithium ascorbate produced the clearest behavioral effect in the tail suspension test. On day 7, mice receiving lithium ascorbate displayed an immobility duration of 175 ± 16.8 seconds, compared with 240 ± 25.5 seconds in the control group. Active mobility was 73 ± 6.8 seconds, compared with 40 ± 8.4 seconds in controls, and passive mobility was 112 ± 9.7 seconds, compared with 80 ± 12.5 seconds. These differences corresponded to an approximately 27% reduction in immobility, an 83% increase in active mobility, and a 40% increase in passive mobility (Table 2).

Group	Primary physiological values		
	Immobilization time (sec)	Active mobility time (sec)	Passive mobility time (sec)
	Day 7 of administration		
1	241±26.4	38±12.4	81±10.5
2	175±16.8*	73±6.8*	112±9.7*
3	240±21.3	37±14.3	83±14.6
4	238±23.4	48±14.8	74±12.7
5	235±16.2	48±16.4	77±14.8
Control	240±25.5	40±8.4	80±12.5
	Day 14 of administration		
1	236±24.1	44±7.6	80±12.7
2	170±16.8*	79±6.8*	111±14.7*
3	246±22.1	35±6.3	79±10.7
4	249±23.5	28±6.9	83±9.2
5	241±24.6	48±8.9	71±11.4
control	248±23.5	38±7.6	74±9.1

Remark: *p < 0.05 compared to control using Student's t-test. 1- Ascorbic acid, 2- Lithium ascorbate, 3- Lithium carbonate + Ascorbic acid, 4- Lithium carbonate, 5- Lithium carbonate + Ascorbic acid, 6 - Control

Table 2. Effect of lithium salts and their combinations on mouse behavior in the tail suspension test (M±SD, n=12)

The effect persisted and modestly increased by day 14. Immobility in the lithium ascorbate group was 170 ± 16.8 seconds, compared with 248 ± 23.5 seconds in the control group, representing a reduction of approximately 31%. Active mobility increased to 79 ± 6.8 seconds, compared with 38 ± 7.6 seconds in controls, corresponding to an approximately 2.1-fold increase. Passive mobility was 111 ± 14.7 seconds in the lithium ascorbate group and 74 ± 9.1 seconds in controls.

The other treatments did not produce comparable statistically significant changes. Ascorbic acid alone showed only a limited directional reduction in immobility after prolonged administration. Lithium carbonate, whether administered alone or in the reported combinations, did not substantially improve tail-suspension behavior under the conditions of the experiment. These observations suggest that the activity

of lithium ascorbate could not be reproduced simply by administering lithium carbonate and ascorbic acid as separate components at the reported doses.

Stress-protective activity was evaluated using a simulated transport-stress procedure . Cages containing six freely moving mice were secured to an orbital shaker and agitated for 24 minutes at 120 revolutions per minute. Blood samples were subsequently collected for the measurement of adrenaline, norepinephrine, and circulating eosinophils (Table 3).

Group	Values		
	Adrenaline (ng/ml)	Norepinephrine (ng/ml)	Eosinophil count (cells/µl)
	Day 7 of administration		
1	18.7±2.4	46.9±9.4	491±47
2	8.1±0.7*	24.3±5.8*	798±69*
3	17.9±2.4	48.3±7.4	453±49
4	19.4±0.9	45.1±10.1	311±58
5	18.5±2.1	43.5±7.6	468±67
Control	18.6±1.4	45.4±6.1	428±62
	Day 14 of administration		
1	20.6±2.7	44.3±7.9	411±67
2	7.6±1.1*	22.7±8.7*	864±81*
3	22.3±2.9	48.7±10.2	410±41
4	21.8±3.1	49.4±8.4	400±68
5	20.1±1.4	45.1±6.8	454±39
Control	21.6±2.3	48.9±7.6	401±81

Table 3. Blood marker values in mice during the simulated transport stress test (M±SD, n=12).

On day 7, the lithium ascorbate group exhibited an adrenaline concentration of 8.1 ± 0.7 ng/mL, compared with 18.6 ± 1.4 ng/mL in controls. Norepinephrine was 24.3 ± 5.8 ng/mL, compared with 45.4 ± 6.1 ng/mL in controls. These differences represented reductions of approximately 56% and 46%, respectively. Circulating eosinophils were higher in the lithium ascorbate group, reaching 798 ± 69 cells/µL compared with 428 ± 62 cells/µL in controls.

The differences became slightly larger after 14 days of treatment. Adrenaline was 7.6 ± 1.1 ng/mL in the lithium ascorbate group and 21.6 ± 2.3 ng/mL in controls, corresponding to an approximately 65% reduction. Norepinephrine was 22.7 ± 8.7 ng/mL, compared with 48.9 ± 7.6 ng/mL in controls, representing a reduction of approximately 54%. The eosinophil count increased to 864 ± 81 cells/µL in the lithium ascorbate group, whereas the control value was 401 ± 81 cells/µL.

Lithium carbonate, ascorbic acid, and their reported combinations did not produce comparable reductions in catecholamine concentrations. Their adrenaline and norepinephrine values generally remained close to those of the control animals at both assessment points. This finding paralleled the behavioral results of the tail suspension test, in which lithium ascorbate was the only treatment to produce a clear and statistically significant reduction in immobility.

The lower catecholamine concentrations observed after simulated transport stress may indicate attenuation of sympathoadrenal activation (Kvetnansky et al., 2013; Kyrou & Tsigos, 2009). Similarly, maintenance of circulating eosinophils may be consistent with a reduction in the physiological stress response because acute stress and glucocorticoid activation can produce eosinopenia. However, circulating catecholamines and eosinophils are influenced by blood-collection timing, handling, circadian variation, assay characteristics, and redistribution between vascular and tissue compartments. These endpoints should therefore be interpreted as indirect markers rather than specific measures of antidepressant action.

Taken together, the behavioral and biochemical results indicate that repeated lithium ascorbate administration was associated with reduced behavioral immobility, increased escape-related activity, lower post-stress catecholamine concentrations, and preservation of circulating eosinophils. The effects were detectable after seven days and were maintained or enhanced after 14 days, suggesting a time-dependent or cumulative response.

Study 2: Effects of Organic and Inorganic Lithium Salts on Open-Field Behavior and Body-Weight Dynamics

The effects of different lithium salts on spontaneous locomotor activity, exploratory behavior, and anxiety-related responses were examined in male rats using the open field test. Sixty outbred white rats, 85 days of age and weighing approximately 220–230 g, were allocated to six groups of ten animals. The groups included an untreated intact group, a vehicle-treated control group, and four experimental groups receiving lithium carbonate, lithium succinate, lithium ascorbate, or lithium orotate.

Before group allocation, the animals were assessed in the open field to characterize baseline emotional reactivity. Experimental groups were subsequently balanced according to body weight. The lithium preparations were administered orally by gastric gavage at a compound dose of 10 mg/kg once daily for seven days before morning feeding. Control animals received physiological saline, whereas intact animals were not subjected to the handling and administration procedures applied to the other groups.

The open field apparatus was a square arena measuring 120 × 120 cm, surrounded by 30-cm walls. The floor was divided into 36 equal squares, and holes were located in 16 central squares. Each observation period included two minutes of exposure in a dark box followed by four minutes in the open field. The recorded parameters were horizontal locomotor activity, vertical locomotor activity, exploration of holes, entries into the central squares, grooming episodes, and fecal-bolus production.

Horizontal locomotor activity was defined as the number of squares crossed, whereas vertical activity was quantified as the number of rearings (Prut & Belzung, 2003). Hole exploration was used as an index of investigatory behavior. Entries into the central area were interpreted as an indicator of reduced avoidance of exposed space, while grooming and defecation were treated as indices of emotional reactivity (Seibenhener & Wooten, 2015). Intergroup comparisons were performed using the Wilcoxon–Mann–Whitney test, with results reported as the mean ± standard error (Table 4).

Groups	HLA	VLA	Holes	Center Entries	Grooming	Boluses
1. control	23.1±2.28	5.44±0.67	4.88±0.72	0	7.63±1.00	2.25±0.25
2. intact	27.0±1.27	7.25±0.37	6.63±0.38	1.16±0.28	6.84±0.42	1.89±0.31
3. Li_2CO_3	23.6±1.11	5.43±0.75	4.75±0.58	0	7.25±0.88	2.13±0.40
4. $C_4H_4Li_2O_4$	29.7±2.54	10.9±0.72*	7.38±0.96*	2.71±0.27*	3.58±0.50*	1.05±0.31
5. $C_6H_7LiO_6$	29.4±2.01	10.3±1.13*	7.63±0.28*	2.13±0.30*	3.50±0.38*	1.15±0.62
6. $C_5H_3LiN_2O_4$	29.1±1.75	9.88±0.77*	7.12±0.54*	2.09±0.14*	3.79±0.16*	1.13±0.40

HLA — horizontal locomotor activity; VLA — vertical locomotor activity (number of rearings); Center Entries — crossing of central squares; Holes — number of holes explored or sniffed; Grooming — number of grooming episodes; Boluses — number of fecal boluses (defecation).

Table 4. Effect of lithium salts on the scope and structure of rat behavior in the Open Field test during subchronic oral administration (M±m, n=10).

Lithium carbonate did not substantially alter open-field behavior relative to the saline-treated control group. Horizontal activity was 23.6 ± 1.11 crossings in the lithium carbonate group and 23.1 ± 2.28 crossings in controls. Vertical activity was also nearly identical, with values of 5.43 ± 0.75 and 5.44 ± 0.67 rearings, respectively. Neither group entered the central squares, and their levels of hole exploration, grooming, and defecation were comparable. Qualitative observations indicated that these animals tended to remain close to the arena walls and exhibited limited exploratory behavior.

In contrast, the three organic lithium salts increased several measures of locomotor and exploratory activity. Horizontal activity reached 29.7 ± 2.54 crossings in the lithium succinate group, 29.4 ± 2.01 in the lithium ascorbate group, and 29.1 ± 1.75 in the lithium orotate group. These values were approximately 26–29% higher than the control value.

The increase in vertical activity was more pronounced. Rats receiving lithium succinate, lithium ascorbate, and lithium orotate exhibited 10.9 ± 0.72, 10.3 ± 1.13, and 9.88 ± 0.77 rearings, respectively, compared with 5.44 ± 0.67 in the control group. Thus, vertical exploratory activity was approximately 1.8- to 2-fold greater after administration of the organic salts.

Hole exploration was also increased. The animals explored 7.38 ± 0.96 holes after lithium succinate, 7.63 ± 0.28 after lithium ascorbate, and 7.12 ± 0.54 after lithium orotate, compared with 4.88 ± 0.72 holes in controls. Although the source report describes these changes as 2.0- to 2.7-fold increases, the tabulated means indicate more moderate increases of approximately 46–56%. The numerical values from the table should therefore be used in preference to the narrative fold-change estimates.

The organic lithium salts also increased entries into the central area. Control animals and lithium-carbonate-treated animals did not enter the central squares, whereas the mean numbers of center entries were 2.71 ± 0.27 for lithium succinate, 2.13 ± 0.30 for lithium ascorbate, and 2.09 ± 0.14 for lithium orotate. Intact animals exhibited 1.16 ± 0.28 center entries. Increased exploration of the center, together with higher locomotor and investigatory activity, was interpreted as a reduction in avoidance and uncertainty in the novel environment.

Grooming was lower in animals receiving the organic lithium salts. The mean numbers of grooming episodes were 3.58 ± 0.50, 3.50 ± 0.38, and 3.79 ± 0.16 in the lithium succinate, lithium ascorbate, and lithium orotate groups, respectively, compared with 7.63 ± 1.00 in the control group. Fecal-bolus production showed a similar directional change, declining from 2.25 ± 0.25 in controls to approximately 1.05–1.15 in the organic-lithium groups. Collectively, these findings indicate that organic lithium salts were associated with increased exploration and reduced autonomic or displacement-related behavioral responses during exposure to a novel arena.

Body-weight dynamics differed markedly among the treatments. Control animals showed essentially no weight gain during the seven-day period, whereas intact animals gained 1.3%. Weight gain was 0.7% in the lithium carbonate group, 2.4% in the lithium succinate group, and 3.2% in the lithium ascorbate group. Lithium orotate was associated with a substantially larger reported increase of 16.4%, corresponding to a change from 220.3 ± 1.67 g to 256.4 ± 5.24 g (Table 5).

Groups	Initial weight (g)	Weight after administration (g)	Weight gain (%)	Gain for the period (g)	Average daily gain (g)
1. Control	221.1±1.12	220.9±3.12	- 0.1	-0.2±0.05	0
2. Intact	222.0±2.24	224.9±2.16	1.3	2.9±0.4	0.36±0.11
3. Li_2CO_3	220.4±2.45	221.9±1.98	0.7	1.5±0.7	0.19±0.09
4. $C_4H_4Li_2O_4$	220.6±1.98	225.9±3.24	2.4	5.3±0.8	0.66±0.10
5. $C_6H_7LiO_6$	221.4±2.08	228.5±3.18	3.2	7.1±0.6	0.89±0.08
6. $C_5H_3LiN_2O_4$	220.3±1.67	256.4±5.24*	16.4	36.1±1.2	4.51±0.56*

(*$p < 0.05$ compared to intact animals according to Student's t-test).

Table 5. Dynamics of weight values and average daily gains in rats during the administration of lithium salts (M±m, n=10)

The magnitude and rapidity of the reported weight increase in the lithium orotate group require cautious interpretation. A 16.4% increase over seven days is considerably larger than the changes observed in all other groups and may reflect increased food intake, fluid retention, differences in measurement conditions, a treatment-related metabolic effect, or an unrecognized data or transcription issue. Replication with systematic measurements of food consumption, water intake, body composition, and fluid balance would be necessary before attributing the change to an anabolic action.

In summary, seven-day administration of lithium succinate, lithium ascorbate, and lithium orotate increased locomotor and exploratory activity and reduced several anxiety-associated or emotionality-related measures in the open field test. Lithium carbonate did not produce comparable effects at the tested compound dose. The findings support the hypothesis that the biological activity of lithium-containing compounds may be influenced by the accompanying anion.

Study 3: Antidepressant-Like Effects of Lithium Ascorbate in the Forced Swim Test

The antidepressant-like effects of lithium salts and lithium-containing combinations were evaluated in adult male Wistar rats using a modified Porsolt forced swim test. The study was conducted in two consecutive stages. During the first stage, the animals received the test compounds once daily for 14 days, and behavioral assessments were performed on treatment days 7 and 14 (Table 6). During the second stage, the persistence of the behavioral effects was evaluated on days 7 and 14 after treatment withdrawal.

The animals were housed under standardized conditions at 21–22°C and had unrestricted access to water and a standard laboratory diet. Test compounds were administered intraperitoneally once daily in a volume of 2 mL per animal. According to the study report, the experimental treatments included ascorbic acid, lithium ascorbate, lithium carbonate, and combinations of lithium carbonate with ascorbic acid. Each experimental group comprised six animals (Table 6).

Group	Dosage, mg/kg	
1	Ascorbic acid	10.00
2	Lithium ascorbate	10.00
3	Lithium carbonate + Ascorbic acid	1.70
	Lithium carbonate	10.00
4	Lithium carbonate + Ascorbic acid	50.00
5	Control	50.00
	Ascorbic acid	10.00
6	Lithium ascorbate	-

Table 6. Dosage calculation by groups

The forced swim apparatus consisted of plastic cylinders measuring 30 cm in height and 10 cm in diameter, filled with water maintained at 25 ± 1°C to a depth of 20 cm. Each test session lasted 10 minutes. Three behavioral states were quantified: climbing, passive swimming, and immobility. Climbing was defined as upward-directed movement of the forelimbs against the cylinder wall (Detke et al., 1995). Passive swimming consisted of movements required for displacement or maintenance of position without upward-directed escape behavior (Porsolt et al., 1977). Immobility was defined as the absence of active movement other than that required to keep the head above the water surface.

Lithium ascorbate produced the most pronounced antidepressant-like behavioral profile among the tested compounds. On treatment day 7, the lithium ascorbate group exhibited a passive swimming duration of 300.83 ± 11.91 seconds and an immobility duration of 194.00 ± 21.25 seconds (Table 7). The corresponding control values were 239.67 ± 29.49 seconds and 291.33 ± 45.29 seconds, respectively. Thus, lithium ascorbate was associated with an approximately 33% reduction in immobility and a 26% increase in passive swimming relative to the control group.

Groups	Day 7 of administration			Day 14 of administration		
	Climbing (s)	Passive swimming time (s)	Immobilization time (s)	Climbing (s)	Passive swimming time (s)	Immobilization time (s)
1	115.17±13.41*	244.83±16.44	240.00±17.96*	126.50±9.77*	249.67±18.28	224.83±20.86
2	105.17±17.13*	300.83±11.91*	194.00±21.25	115.17±18.26	317.50±13.03*	166.50±22.21
3	119.33±9.18*	224.17±16.23	256.50±23.15	120.50±9.27*	230.17±17.35	249.67±24.22
4	70.33±21.87	240.50±37.59	289.17±52.00	67.00±51.19	247.33±37.13	287.00±49.69
5	109.83±15.75	245.17±30.38	245.00±36.29	110.50±14.79*	246.67±30.83	245.33±36.39
6	69.00±22.49	239.67±29.49	291.33±45.29	65.00±20.86	233.50±28.88	302.83±43.29

*p < 0.05 compared to control (Student's t-test).

Table 7. Effect of lithium salts and their combinations on rat behavior in the Porsolt test (M±m, n=6)

The effect became more pronounced after 14 days of administration. Passive swimming increased to 317.50 ± 13.03 seconds, compared with 233.50 ± 28.88 seconds in the control group, while immobility decreased to 166.50 ± 22.21 seconds, compared with 302.83 ± 43.29 seconds in the control group. These values corresponded to an approximately 36% increase in passive swimming and a 45% reduction in immobility. The duration of climbing was not substantially increased, indicating that the reduction in immobility was primarily accompanied by a redistribution of behavior toward sustained swimming rather than a generalized increase in vigorous escape activity.

Lithium carbonate demonstrated a substantially weaker behavioral effect. On day 7, the behavioral parameters in animals receiving lithium carbonate were generally similar to those of the control group. By day 14, only modest changes were observed, including an approximately 5% reduction in immobility and small increases in climbing and passive swimming. These findings suggest that, at the tested dose and under the conditions of this experiment, lithium carbonate did not reproduce the magnitude of the behavioral effect observed with lithium ascorbate.

Ascorbic acid also modified the structure of swimming behavior. Its administration was associated with reduced immobility and increased climbing, particularly during the active phase of the test. The behavioral pattern produced by ascorbic acid differed from that observed with lithium ascorbate: ascorbic acid predominantly increased climbing behavior, whereas lithium ascorbate produced a greater increase in passive swimming (Detke et al., 1995). This distinction may indicate that the compounds affected different components of the behavioral response, although the forced swim test alone cannot establish the underlying neurochemical mechanisms (Commons et al., 2017).

The persistence of the effects was assessed after treatment discontinuation (Table 8). Seven days after lithium ascorbate withdrawal, passive swimming remained elevated at 292.83 ± 9.47 seconds, compared with 232.17 ± 26.95 seconds in the control group. Immobility remained reduced at 196.83 ± 18.27 seconds, compared with 298.83 ± 44.05 seconds in controls. Fourteen days after withdrawal, the lithium ascorbate group continued to demonstrate significantly greater passive swimming and lower immobility than the control group, although the magnitude of the difference was smaller than that observed during active administration. At this time point, passive swimming was 252.67 ± 15.65 seconds and immobility was 247.33 ± 20.19 seconds, compared with 236.50 ± 25.02 seconds and 290.67 ± 37.53 seconds, respectively, in the control group.

	Day 7 of withdrawal			Day 14 of withdrawal		
	Climbing (s)	Passive swimming time (s)	Immobilization time (s)	Climbing (s)	Passive swimming time (s)	Immobilization time (s)
1	70.50±9.54*	230.17±14.95*	300.00±13.64*	69.83±21.14	235.67±9.48*	295.00±26.04*
2	110.67±13.13*	292.83±9.47*	196.83±18.27*	100.83±6.05*	252.67±15.65*	247.33±20.19*
3	69.67±21.32	231.50±15.02*	286.83±31.22	70.50±26.88	233.17±23.32	297.17±30.07
4	86.50±15.06*	254.33±33.01	259.67±46.61	78.83±22.44	242.50±30.20	261.67±31.82
5	87.50±20.21	255.67±22.77	257.33±36.42	78.00±18.90	243.83±23.02	278.17±31.95
6	70.83±22.66	232.17±26.95	298.83±44.05	72.17±18.31	236.50±25.02	290.67±37.53

*p < 0.05 compared to control (Student's t-test).

Table 8. Rat behavior in the Porsolt test following the withdrawal of lithium salts and their combinations (M±m, n=6)

By contrast, the effects associated with ascorbic acid declined after treatment withdrawal, and the animals' behavior gradually approached that of the control group. This difference suggests that lithium ascorbate produced a more persistent behavioral effect than ascorbic acid alone. Nevertheless, repeated exposure to the forced swim procedure may itself have resulted in habituation or adaptation, as reflected by changes in the behavior of control animals across successive assessments (Commons et al., 2017).

Overall, repeated administration of lithium ascorbate produced a consistent antidepressant-like behavioral response in the modified forced swim test, characterized by reduced immobility and increased passive swimming. The response was evident after seven days, became stronger after 14 days, and remained partially detectable for up to 14 days after withdrawal. Lithium carbonate produced only minor changes under the same experimental conditions.

Conclusion

Across three preclinical experiments, lithium ascorbate produced a consistent pattern of antidepressant-like and stress-attenuating effects in rodents. Repeated administration reduced immobility and increased active or sustained coping behavior in the tail suspension and forced swim tests. In the open field, lithium ascorbate increased exploratory activity and center entries while reducing grooming and defecation. Following simulated transport stress, it was associated with lower adrenaline and norepinephrine concentrations and higher circulating eosinophil counts than the control condition.

The behavioral effects were detectable after seven days of administration and were generally maintained or enhanced after 14 days. In the forced swim experiment, part of the response remained observable after treatment withdrawal, although repeated testing and adaptation to the procedure may have contributed to this finding.

Under the conditions of the reported studies, lithium ascorbate showed a more consistent effect than lithium carbonate or ascorbic acid alone. However, the experiments do not establish the clinical superiority of lithium ascorbate or demonstrate pharmacological synergy between lithium and ascorbate.

Comparisons among lithium salts were limited by differences in elemental lithium exposure, study design, species, administration route, and treatment duration.

The findings should therefore be interpreted as preliminary evidence that lithium ascorbate modifies behavioral coping, exploratory behavior, and physiological stress responses in rodents. They do not demonstrate efficacy for depression, anxiety, or other psychiatric disorders in humans.

Discussion

Across three preclinical experiments, lithium ascorbate produced a broadly consistent pattern of behavioral and physiological effects. Repeated administration reduced immobility in the tail suspension and forced swim tests, increased active or sustained coping behavior, enhanced exploratory activity in the open field, and attenuated physiological responses to simulated transport stress. The convergence of these findings across mice and rats provides preliminary evidence of antidepressant-like and stress-attenuating activity.

The effects were not restricted to a single behavioral endpoint. In both the tail suspension and forced swim tests, lithium ascorbate reduced immobility after seven days, with generally greater effects after 14 days. In the forced swim test, the reduction in immobility was accompanied primarily by increased sustained swimming rather than a marked increase in climbing. This pattern argues against a simple nonspecific increase in vigorous motor activity. Nevertheless, these tests cannot independently distinguish antidepressant-like activity from changes in arousal, locomotion, stress reactivity, fatigue, or thermoregulation and should therefore be interpreted together with complementary behavioral and physiological measures.

The open field findings support the interpretation that lithium ascorbate modified responses to novelty and stress. Treated rats showed greater horizontal and vertical activity, increased hole exploration and center entries, and reduced grooming and defecation. This profile is compatible with increased exploratory motivation and reduced defensive or anxiety-associated behavior. However, open-field activity is not specific to anxiolysis and may also reflect psychomotor stimulation or altered novelty responsiveness. Replication using assays less dependent on locomotor activity is therefore required.

Lithium ascorbate was also associated with lower adrenaline and norepinephrine concentrations and higher circulating eosinophil counts following simulated transport stress. These findings may reflect attenuation of sympathoadrenal and systemic stress activation. However, catecholamine and eosinophil measurements are sensitive to sampling time, handling, circadian variation, assay conditions, and cellular redistribution. Because unstressed baseline groups were not clearly reported, it is not possible to determine whether lithium ascorbate prevented stress-induced changes, accelerated recovery, or altered baseline physiology.

The effects generally increased between days 7 and 14, suggesting a time-dependent pharmacodynamic response. In the forced swim experiment, part of the behavioral effect remained detectable after treatment withdrawal. This persistence could indicate longer-lasting biological adaptation, but repeated testing may also have influenced behavior through habituation, learning, fatigue, or sensitization. Future studies

should use separate cohorts for different assessment times or apply appropriate repeated-measures analyses.

Lithium carbonate produced weaker or negligible effects in several comparisons, whereas organic lithium salts showed greater behavioral activity in the open field. These findings raise the possibility that the accompanying anion influences the pharmacological profile of lithium-containing compounds through differences in absorption, distribution, cellular transport, metabolism, or lithium-independent biological activity. However, the experiments do not permit a definitive ranking of the salts because equal compound doses were used without clearly matching elemental lithium exposure. The observed differences may therefore reflect unequal lithium doses, anion-specific effects, pharmacokinetic differences, or a combination of these factors.

Differences in species, strain, administration route, treatment duration, and experimental design preclude quantitative pooling. The findings should therefore be regarded as qualitative convergence across complementary models rather than as a combined estimate of efficacy. Moreover, immobility in the forced swim or tail suspension test is not equivalent to human depression, and open-field center exploration is not equivalent to clinical anxiety. The results support the use of terms such as “antidepressant-like,” “stress-attenuating,” and “anxiety-related,” but not claims of clinical antidepressant or anxiolytic efficacy.

Data availability

All source data for this study are available upon reasonable request.

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Ethics declarations

This research was conducted according to the GLP/OECD guidelines (OECD, 1998a). These studies were reviewed at the meetings of the Bioethics Commission for compliance with the “Three R’s” principles and Directive 2010/63/EU.

Competing interests

The experiments were paid for by Normopharm LLC, Russia.

Abbreviations

ANOVA — analysis of variance
C57BL/6J — C57BL/6J inbred mouse strain
GLP — Good Laboratory Practice

GMP — Good Manufacturing Practice
HLA — horizontal locomotor activity
HPA axis — hypothalamic–pituitary–adrenal axis
i.p. — intraperitoneal
Li — lithium
Li_2CO_3 — lithium carbonate
OECD — Organisation for Economic Co-operation and Development
SD — standard deviation
SEM — standard error of the mean
TST — tail suspension test
VLA — vertical locomotor activity
WHO — World Health Organization

The following may also be included, depending on the final wording:

CNS — central nervous system
FST — forced swim test
OF test or **OFT** — open field test
rpm — revolutions per minute
GOST — Russian national standard
M ± SD — mean ± standard deviation
M ± SEM — mean ± standard error of the mean

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