Effect Of Therapeutic Lithium Carbonate Doses On Rat Brain Content Of Mood Regulating Neurotransmitters

Abdel-Hamid, NM, El-Shaer, SS, Gad, A

Ex-Dean of Faculty of Pharmacy, Kafrelsheikh University, Egypt; Tel: +201227300491; Fax: +20473215768
E-mail: nabilmohie@yahoo.com

Biochemistry Department, Faculty of Pharmacy, A-Azhar University, Cairo, Egypt
Pharmacology Department, National Organization of Drug Control and Research, Giza, Egypt.

Abstract: The anti-depressant value of lithium on rat brain tissue was studied at different treatment periods to explore its impact on brain neurotransmitters related to mood disorders (dopamine and histamine), precurors with further highlighting on minerals implicated in their synthesis (magnesium and calcium). We examined a therapeutic dose (75 mg/kg/d) on four groups of rats, 6/each, grouped according to duration into, control group; given saline (group 1), acute study group; given single dose, left for 2 hours (group 2), sub-acute study group; given a dose each 3 days for 2 weeks (group 3) and a chronic study group; given a dose each 3 days for a month (group 4). Brain content of dopamine and histamine was significantly decreased on acute treatment, but increased for dopamine and decreased for histamine after 2 weeks while it flipped for each of them after one month of administration. Gama aminobutyrate (GABA), glycine and histidine were significantly decreased after each period of treatment while phenyl alanine was significantly decreased only after a month of treatment. These changes occurred with increased magnesium and decreased calcium brain content on both acute and sub-acute treatment. Brain content of both of them was decreased on chronic treatment. These actions reveal that lithium has a remarkable effect on brain neuronal function which probably postulates an interesting impact of lithium treatment in shifting amino acids and needed minerals in direction of improving the mood, through neurotransmission homeostasis. List of abbreviations: 5HT: serotonin; AA: arachidonic acid; Ach: acetylcholine; AD: Alzheimer disease; BD: bipolar disorder; BDNF: brain-derived neurotrophic factor; cAMP: cyclic adenosine monophosphate; CdK5: cyclin-dependent kinase 5; ER: endoplasmic reticulum; GABA: gamma-Aminobutyric acid; GSK3: Glycogen synthase kinase 3; HDAC: Histone deacetylase; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; IP3: inositol 1, 4, 5-trisphosphate; NMDA: N-methyl-D-aspartic acid; PAI-1: plasminogen activation inhibitor-1; PD: Parkinson's disease; SNC: substantianigra pars compacta; TBARS: Thiobarbituric acid reactive substances; TNF-α: Tumor necrosis factor alpha.

Key words: Lithium carbonate; Brain tissue; Bipolar Disorders; Neuroprotective; Neurotransmitters; Amino acids; Dopamine; Histamine; Magnesium, Calcium.

1. Introduction
Lithium salts have been widely used in different fields of medicine for more than sixty years, specially in psychiatry [1-5]. There have been some reports about promising outcomes concerning the possibility of its application in neurology [6, 7]. Lithium is largely known for its usage in the treatment of bipolar disease. The mechanism of lithium’s mood-stabilizing activity has not been clarified, in part because lithium exerts its effects on a large scale of cellular functions; for example, it inhibits inositol production, affects the protein kinase C signaling pathway and inhibits glucogen synthase kinase 3 (GSK3) [8-10]. Moreover, various studies in cellular and animal models have demonstrated that lithium can suppress neurodegeneration resulted from different sorts of insults, such as Alzheimer disease and amyloid-B peptide [11], glutamate excite toxicity and ischemia [12]. Consistent with its protective actions, lithium treatment enhanced Bcl-2 expression but decreased Bax and p53 expression in rat cerebellar granule cells under glutamate challenge, thus prohibiting caspase activation [13]. Acute and chronic treatment of bipolar disorder (BD) patients with lithium carbonate has been accompanied by up-regulation of particular biological cascades related to neuro-protection. Lithium treatment significantly increased plasma levels of brain-derived neuro-trophic factor (BDNF), with a motivating efficacy on response to treatment. Also, lithium modifies other remarkable biological processes related to inflammation and oxidative stress [14], besides, lithium treatment of acute mania episodes was accompanied by a worthy reduction of pro-oxidative stress markers, that are thiobarbituric acid reactive substances (TBARS) [15]. Additionally, lithium has been proved to be engaged in remyelination and axonal regeneration [16]. BD patients who reacted with lithium treatment in a good response also had a serious reduction in plasma levels of tumor necrosis factor alpha (TNF-α); in contrast, BD patients who did not quiet respond to lithium showed a significant increase in TNF-α levels [17]. More and above that, lithium can restore the balance between the production of interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) in monocytes of bipolar patients in vitro; this effect is similar to those observed in vivo [18]. There are some other mechanisms by which lithium has been shown to act intracellularly. These include modification of cyclic adenosine monophosphate (cAMP)-mediated signal transduction [19-21]; lessening in the arachidonic acid (AA) cascade [22]; negative regulation of the Smad3/4-transcription factor and protein levels of plasminogen activator inhibitor-1 (PAI-1); and induction of neurogenesis [23]. Furthermore, lithium has been indicated to motivate the survival pathway, MEK/ERK [24]; increase transcription factor β-catenin levels [25]; and regulate autophagy via inositol inhibition and...
reduction of inositol 1, 4, 5-trisphosphate (IP3) levels [26]. An ample evidence elucidated that dysfunction of serotoninergic neurotransmission in CNS is involved in the evolution of anxiety, depression and memory disorders [27]. The impact of lithium on serotonin metabolism is established, it increases brain tryptophan, 5-HIAA and 5-HT levels, as well as increased synthesis rate of brain serotonin [28]. Lithium carbonate has been noted to alter GABA levels, possibly decreasing impulsive and aggressive behavior by increasing availability and efficacy of GABA [29]. A worthy notable that lithium affects memory by interaction with acetylcholine (Ach), N-methyl-D-aspartate (NMDA), serotonin (5HT), dopamine (DA), neurotrophic factors, prostaglandins and kinases in distinct brain areas [30]. These observations propose an influential function of lithium in synaptic plasticity and cognition tuning. Amino acids can serve as the most powerful compounds in the treatment of neurological diseases in the upcoming years. Amino acids maintain regeneration and reorganization processes of neurons [31]. Changes of these amino acids neurotransmitters have been notified in many neurological and psychiatric disorders such as depression, Alzheimer’s disease, schizophrenia, and epilepsy [32-36]. As an outcome of these observations, this study was conducted to clarify lithium carbonate impact on the metabolic pathways at different treatment durations and assess brain content of neurotransmitters and amino acids/neurotransmitters exchange at brain tissue, to add further explanation of neuro-protective role of therapeutic doses of lithium carbonate.

2. Materials and methods

2.1. Animals
A total of twenty four male Swiss albino rats, weighting between 150-200 g each, were used in this study. The animals were purchased from Faculty of Veterinary Medicine, Zagazig University, Egypt. Animals were kept for two weeks for acclimatization under standard laboratory conditions with temperature at 23±2°C, and 12:12 light/dark cycle. The animals were allowed free access to food and water. The standard guidelines were used in handling the experimental animals and we complied with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Experimental Design
Rats were randomly classified into 4 experimental groups; Group 1: Served as control, rats received saline (1 ml; i.p.). Group 2: Rats received single dose of lithium carbonate (75 mg/kg; i.p.), used for the study of the acute effect of lithium carbonate, then sacrificed after 2 hrs. Group 3: Rats received lithium carbonate (75 mg/kg; i.p. sub-acute study) for two consecutive weeks. Group 4: Rats received lithium carbonate (75 mg/kg; i.p, chronic study) for 4 weeks. The animals treated for two weeks and one month were injected in 1st and 4th day of the week, thus the animals treated for two weeks received 5 doses and those for a month received 10 doses. The animals treated for two weeks were sacrificed on the third day of the last injection; while those treated for a month were sacrificed on the fourth day of the last injection. After scarification of rats, brains were carefully removed, blotted and chilled. The tissue content of brain neurotransmitters (dopamine and histamine), some free amino acids (γ-aminobutyrate, glycine, histidine; histamine precursor, phenyl alanine; dopamine precursor and tyrosine) as well as calcium and magnesium were estimated.

2.3. Chemicals
All chemicals were of analytical grade, obtained from Sigma-Aldrich, USA.

2.4. Methods
Estimation of free amino acids in brain tissue extract
The extraction of free amino acids from brain tissue was made according to steps reported before [37], as follows: 1 g of brain tissue homogenate was triturated with 5 ml of 80% ethanol at 4°C. The mixture was centrifuged at 3000 rpm for 5 minutes. The clear supernatant was subjected for amino acid determination (glycine, γ-aminobutyrate, histidine, phenyl alanine and tyrosine) using amino acid auto analyzer (Techneicon, Model R.120).

Estimation of Dopamine
It was estimated in brain homogenate according to the method described elsewhere Schlumpf, Lichtensteiger, Langemann, Waser and Hefti [38]. At the end of experiment rats were sacrificed, whole brain was dissected out and separated. One gm of frozen tissue was homogenized in 12ml HCl–butanol for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant (9.6 ml) was pipetted and added to centrifuge tube containing 24 ml heptane and 3 ml 0.1 M HCl. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (2.4 ml) was then taken for Fluorometric DA assay. All steps were carried out at 0°C. Dopamine was determined spectrofluorimetrically. The fluorescent compound produced was measured by Schimadzu spectrofluorometer Rf 510, at excitation wavelength of 330 nm and emission wavelength of 375 nm.

Estimation of Histamine
One g of brain tissue was triturated with 4 ml of 20% trichloroacetic acid and the mixture was left aside for 3 hours on cold. Then the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was separated and shaken with equal volume of ether for 3 min to remove excess trichloroacetic acid, and then the ether phase was discarded. To the aqueous phase, 1 g of ion-exchange resin was added and set aside for 10 minutes with regular shaking, then centrifuged for 5 min at 3000 rpm. and then the supernatant was separated and preserved at 4°C for 5 min. Then, 0.85 ml of clear supernatant was diluted with equal volume of 1 M HCl. Histamine was assessed spectrofluorimetrically using Schimadzu spectrofluorometer Rf 510, at excitation wavelength of 360 nm and emission wavelength of 450 nm[39].
Estimation of calcium and magnesium
We made many dilutions to fit the scale of the atomic absorption spectrophotometer (Schmidazu). So, 250 mg of brain tissues were homogenized with 12 ml of 0.1 N HCl and 4 ml of 10% trichloroacetic acid, the mixture was centrifuged at 2000 rpm for 3 min, then 0.1 ml of clear supernatant was diluted to 10 ml by 0.1 N HCl. Firstly, magnesium was determined. These samples were used for measuring calcium after addition of 1 ml lanthanum chloride 1% to each tube (using magnesium lamp). Parker, Humoller and Mahler [40]

2.5. Ethical Approval
All procedures performed in this study were in accordance with the ethical standards of the University of Al-Azhar Research Committee (UARC), which are in accordance with the Helsinki declaration 2013; saved in the committee archive at its turn and date.

2.6. Statistical analysis
The statistical analysis of data was done using Graph Pad Prism 5 (Graph pad Software, San Diego California, USA). The results were expressed as means ± S.E.M. differences between the mean values for individual groups were assessed by one way ANOVA.

3. Results
Variations in dopamine content after lithium carbonate treatments After two hours of lithium administration to rats, dopamine content was reduced down. Administration of lithium to rats for two weeks produced a slight but significant (P< 0.05) increase in brain dopamine content to 0.94±0.02μg/g tissue. While after a month of treatment, the mean value of dopamine was significantly decreased (Figure 1). Variations in histamine content after lithium carbonate treatments After two hours of lithium administration to rats, histamine content was slightly reduced. Administration of lithium to rats for two weeks produced a significant decrease (P< 0.001) in brain histamine content. However, after a month of treatment with lithium carbonate, the mean value of histamine was significantly increased (Figure 2). Variations in magnesium content after lithium carbonate treatments Lithium carbonate administration caused a significant increase in magnesium content after two hours and after two weeks. However after a month of administration of lithium carbonate, magnesium content was slightly reduced to (Figure 1). Variations in calcium content after lithium carbonate treatments Lithium carbonate administration caused a significant decrease in brain calcium content after two hours and after two weeks, as well as after a month of administration of lithium carbonate (Figure 2). Variations in studied amino acids content after lithium carbonate treatments A significant decrease in brain content of γ-aminobutyrate (GABA) and histidine (histamine precursor) was observed throughout the three time periods (p<0.001), as well as, the neuroinhibitory amino acid, glycine was significantly decreased till reached the least values on longer treatment (one month) while phenyl alanine (dopamine precursor) was significantly decreased only after a month of treatment and tyrosine showed no significant decrease throughout the three periods (Figure 3).

**Fig (1):** The effect of lithium carbonate therapeutic dose (75 mg/kg) on dopamine and magnesium contents in rat brain tissue extract after 2 hrs, 2 weeks and one month of oral administration (Values are expressed as mean ± SE and are considered significant than control group at P < 0.05*).
4. Discussion
The treatment with therapeutic doses of lithium carbonate (75 mg/kg, i.p) caused a significant increase in dopamine content after two weeks. This finding indicated that lithium possibly modulated the function and expression of transcription, factor activator protein 1 (AP-1) in the rats [41]. It was reported that lithium influenced extracellular concentration of dopamine in rat brain, where a decrease in the dopamine concentration in prefrontal cortex [42] and an increase in striatum [43] after the intragastric administration of lithium for two weeks. Reduction of dopamine turnover might have a role in blocking, delaying, or reducing the intensity of the switch process. Lithium mediates glycogen synthase kinase-3b (GSK-3b) inhibition, which may be related to neuroprotective effects against glutamate induced toxicity. Reportedly, the co-
One month of treatment could be parallel with increased content of histamine that indicates a possible activation of histamine synthesis in the brain, by activating histidine decarboxylase [59, 60]. The acute treatment of lithium caused a significant increase in magnesium content after two hours and also after two weeks of administration to rats; however there was a significant reduction in calcium content. Mg$^{2+}$ and Li$^+$ ions have been found to compete for binding-site of some enzymes [61]. Low serum Mg$^{2+}$ levels are associated with several neurological diseases such as migraine, depression, epilepsy and Alzheimer disease (AD) [62-65]. The negative relationships between calcium and lithium were reported. In ovariectomized rats fed zinc-deficient diet, markedly enhanced lithium in bone was associated with a remarkable calcium decline [66]. A case report study concluded that in a lithium-treated patient, a decrease in Ca$^{2+}$ was found [67]. Calcium cytotoxicity has been approved for about 35 years ago. There are some theories elucidate the mechanism of excitotoxicity of Ca$^{2+}$, and all of them emphasize the higher than given threshold cytoplasmatic calcium level as a fundamental factor which enters the cell into the apoptotic pathway [68]. The elevated Ca$^{2+}$ level within the brain tissue in Se treated animals may propose the higher potential for excitotoxicity development; however, this observation needs further studies on cellular level. Lithium actions on the phosphatidylinositol (PI) signaling pathway, for example [69], leads to a reduction in levels of inositol-1,4,5, triphosphate (IP3), which considered as an essential stimulator for intracellular calcium levels [70]. IP3 typically mediates calcium release from the endoplasmic reticulum (ER), the primary site for protein synthesis, folding, trafficking, with additional roles in calcium signaling regulation [71]. Therefore, Lithium’s reduction of IP3 levels inhibits calcium release from the ER, with effects on neuronal functioning [72]; this involves reduction in the activity of the calcium-dependent protein calpain and calpain-mediated activation of pro-apoptotic cyclin-dependent kinase 5 (Cdk5), leading to reduced cellular death [51].

**Conclusion**

Lithium carbonate has a serious role in managing BD, apparently, through conversion of some amino acids into their analogical stimulatory neurotransmitters on both acute and chronic treatments, in addition to handling the level of inhibitory amino acids as GABA and glycine in mood equilibrium trend, controlling neuro-transmission and tissue stabilization.

**Ethical approval and consent to participate:** All procedures performed in this study were in accordance with the ethical standards of the Al-Azhar University Research Committee and with the Helsinki declaration; no formal ethical review was required.

- **Consent for publication:** Not applicable
- **Availability of data and material:** All data generated or analyzed during this study are included in this article
- **Competing interests:** "The authors declare that they have no competing interests"
• **Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

• **Authors’ contributions:** All authors read and approved the final manuscript.

**Author Profile**

• **Name:** Nabil Mohie Abdel-Hamid.

• **Current Position:** Professor of Cancer Biology at Department of Biochemistry, Ex- and First Dean and Founder of Faculty of Pharmacy, Kafrelsheikh University, Egypt.

• **National and International Memberships:**
  - **Member of:** The Higher Consulting Organization of Egyptian Scientists Council.
  - **Deputy of Director of:** The Egyptian Association of Cancer Research.
  - **Member in:** The Arab Evaluators for Scientific Degree Promotions.
  - **Member in:** The Egyptian Evaluators for Scientific Degree Promotions.

• **Research Interest:** Cancer Biology, Principal Instructor and interested in Hepatocellular Carcinoma Research (Both Early Detection, Looking for New Sensitive and Specific Markers and Therapeutic Perusal), as New Trends in Management of Hepatocellular Carcinoma, Including Chemo and Radio Sensitization to Cancer Therapy.

**References:**


for an association between tumor necrosis factor-
alpha levels and lithium response, Journal of affective
disorders, 143 (2012) 148-152.

De Wit, C. Ruwhof, G.W. Akkerhuis, W.A. Nolen,
H.A. Drexhage, An imbalance in the production of
IL-1β and IL-6 by monocytes of bipolar patients:
restoration by lithium treatment, Bipolar disorders, 9

H.K. Manji, G. Chen, The role of the extracellular
signal-regulated kinase signaling pathway in mood
7311-7316.

psychopharmacology, Clinical neuroscience research,

[21] R.S. Jope, A bimodal model of the mechanism of

[22] S.I. Rapoport, F. Bosetti, Do lithium and
anticonvulsants target the brain arachidonic acid
cascade in bipolar disorder?, Archives of General

[23] R. Hashimoto, N. Takei, K. Shimazu, L. Christ, B.
Lu, D.-M. Chuang, Lithium induces brain-derived
neurotrophic factor and activates TrkB in rodent
cortical neurons: an essential step for neuroprotection
against glutamate excitotoxicity, Neuropharmacology,

inhibits Smad3/4 transactivation via increased CREB
signaling activity induced by enhanced PKA and AKT
signaling, Molecular and Cellular Neuroscience, 37

the brain for lithium inhibition of glycogen synthase

[26] D.J. Klionsky, K. Abdelmohsen, A. Abe, M.J.
Abedin, H. Abdeliovich, A. Acevedo Arozena, H.
Adachi, C.M. Adams, P.D. Adams, K. Adeli,
Guidelines for the use and interpretation of assays for
monitoring autophagy, Autophagy, 12 (2016) 1-222.

[27] M. Banasr, G. Chowdhury, R. Terwilliger, S.
Newton, R. Duman, K. Behar, G. Sanacora, Glial
pathology in an animal model of depression: reversal of
stress-induced cellular, metabolic and behavioral
deficits by the glutamate-modulating drug rituxole,

[28] A. Serretti, R. Lilli, L. Mandelli, C. Lorenzi, E.
Smeraldi, Serotonin transporter gene associated with
lithium prophylaxis in mood disorders, The

Akiyama, Y. Nakazato, M. Mikuni, Changes in density
calcium- binding- protein- immunoreactive
GABAergic neurons in prefrontal cortex in schizophrenia and bipolar disorder, Neuropathology, 28 (2008) 143-150.

Sharifabad, A. Hoseini, M. Pourghorban, M.
Aghsami, S. Gholizadeh, M. Abdollahi, A. Roghani,
Time course effects of lithium administration on spatial memory acquisition and cholinergic marker
expression in rats, DARU Journal of Pharmaceutical
Sciences, 17 (2015) 113-123.

[31] I. Morales, L. Guzman-Martinez, C. Cerda-Troncoso,
G.A. Farias, R.B. Maccioni, Neuroinflammation in
the pathogenesis of Alzheimer's disease. A rational
framework for the search of novel therapeutic
approaches, Front Cell Neurosci, 8 (2014) 112.

glutamate synapse in neuropsychiatric disorders.
Focus on schizophrenia and Alzheimer's disease, Prog

[33] Y.V. Tcherkas, L.A. Kartsova, I.N. Krasnova,
Analysis of amino acids in human serum by isotropic
reversed-phase high-performance liquid chromatography with electrochemical detection,

[34] T. Sumiyoshi, A.E. Anil, D. Jin, K. Jayathilake, M.
Lee, H.Y. Meltzer, Plasma glycine and serine levels
in schizophrenia compared to normal controls and
major depression: relation to negative symptoms, Int J

[35] K. Hashimoto, A. Sawa, M. Iyo, Increased levels of
glutamate in brains from patients with mood

[36] O.Y. Nagasawa M, Kurata K, Otsuka T, Yoshida J,
Tonomaga S, Furuse M. Furuse., Hypothesis with
abnormal amino acid metabolism in depression and
stress vulnerability in Wistar Kyoto rats, Amino
Acids 2012 (2012).

studies of diazepam (valium) on rabbit brain

[38] M. Schlumpf, W. Lichtensteiger, H. Langemann, P.G.
Waser, F. Hefti, A fluorometric micromethod for the
simultaneous determination of serotonin,
noradrenaline and dopamine in milligram amounts of
brain tissue, Biochemical pharmacology, 23 (1974)
2437-2446.


**Figure legends:**

**Fig 1:** The effect of therapeutic doses of lithium carbonate (75 mg/kg) on dopamine and magnesium contents of rat brain tissue extract after 2 hrs, 2 weeks and one month of oral administration (Values are expressed as mean ± SE and are considered significant than control group at P < 0.05*).

**Fig 2:** The effect of therapeutic doses of lithium carbonate (75 mg/kg) on histamine and calcium contents in rat brain tissue extract after 2 hrs, 2 weeks and one month of oral administration (Values are expressed as mean ± SE and are considered significant than control group at P < 0.05*).

**Fig 3:** The effect of therapeutic doses of lithium carbonate (75 mg/kg) on glycine, γ-aminobutyrate, histidine, phenylalanine and tyrosine contents in rat brain tissue extract after 2 hrs, 2 weeks and one month of oral administration (Values are expressed as mean ± SE and are considered significant than control group at P < 0.05*).