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Research Article

Can Bipolar Disorders be Treated by Stimulating Neurite Growth with Hydroxydase lithium and Magnesium Rich Water?

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Abstract:

Background/Aim: Bipolar (BD) and depressive disorders (DD) are common psychiatric conditions marked by reduced neuronal cells and impaired neurite growth, leading to disrupted neuronal connectivity and symptoms characteristic of BD or DD. Current treatments poorly address neuronal damage. First-line treatments like lithium (Li) stabilize mood swings but high doses cause side effects. Magnesium (Mg), through a distinct mechanism, partially stabilizes mood. Studies on synergistic effects of Li and Mg are scarce. We evaluated the neurite growth properties of one of the richest source of bioavailable lithium (9737 µg/L) and magnesium (243 mg/L) on the growth of neurones and neurites in vitro.

Materials and Methods: Commercially available rat cortical neurone cultures were exposed on day 1 to 1%, 3%, 5% Hydroxydase water, or to the same volumes of Li-carbonate solution (20 µg/mL) as positive control, or culture medium containing no lithium as negative control. Number of neurites per cell and mean neurite length were analysed on days 1, 3, and 7 using microscopic imaging.

Results: The test substances showed no cytotoxic effects. Li-carbonate had no significant effect on neuronal growth, neurite number or length. Hydroxydase water increased neuronal cell numbers (+11.54%) and enhanced neurite growth (+37%) and elongation (+47%) per cell by day 7. These effects were observed across all concentrations and began as early as day 1.

Conclusion: Hydroxydase water significantly enhances neuronal differentiation, neurite sprouting, and elongation, suggesting improved neuronal connectivity and neurotransmission in vivo. The synergistic action of bioavailable Li^+ and Mg^{2+} presents a natural and effective complement to synthetic drugs for BD and DD treatment.

Key words: neuronal cell; hydroxydase; neurite sprouting; neurite length; lithium; magnesium; depression; bipolar disorders

Abbreviations

BD : (bipolar Disorder)

DD : (Depressive disorder)

BDNF : (brain-derived neurotrophic factor)

HPA : (hypothalamic-pituitary-adrenal)

GABA : (Gamma-aminobutyric acid)

NMDA : (N-methyl-D-aspartate)

Introduction

Bipolar disorders (BD) affect approximately 5% of the global population. Onset typically occurs in late adolescence or early adulthood, with equal prevalence among males and females. Acute or chronic depressive disorders (DD) concern 5-10% of the population, with onset frequently in the mid-twenties [1]

It has been clearly established that both BD and DD are associated with reduced neurogenesis, particularly in the hippocampus [2].The cause(s) and physiopathology of these diseases are not well understood, but chronic stress, specific traumatic events, and certain genetic factors are considered contributors that trigger neuronal degeneration and the disease [3].Reduced numbers of neurites in brain regions such as the prefrontal cortex and hippocampus, along with fewer neurites per neuron, shorter neurite length, and underdeveloped glial cells, are observed in BD and DD patients [4]. These neuronal changes contribute to impaired synaptic connectivity, poor neurotransmission, and, consequently, impaired brain

functioning [5]. BD and DD also involve dysregulation of brain-derived neurotrophic factor (BDNF), which plays a key role in neuronal survival and synaptic plasticity [6]. Chronic inflammation, oxidative stress, and hypothalamic-pituitary-adrenal (HPA) axis dysfunction further exacerbate these impairments, leading to disruptions in serotonin, dopamine, and glutamate neurotransmitters [7].

Understanding these neuronal changes has therapeutic implications. Interventions aim to enhance and stabilize neurotransmission, as well as promote neurogenesis and neurite outgrowth [8]. Antidepressants, mood stabilizers, and lifestyle modifications, such as exercise, help mitigate some cognitive and emotional deficits in BD and DD, but no specific treatment directly addressing neuronal damage is available [9,10].

The most used and effective treatment is Li, which stimulates BDNF production and improves synaptic plasticity. However, there is little evidence that Li promotes neuronal and neurite growth [11,12]. While neuroprotective properties of Li are recognized, its direct impact on neurite outgrowth is inconsistent, likely due to the difficulty of evaluating neuronal or neurite growth in BD patients. Similarly, magnesium (Mg) may help promote neuronal health and minimize BD symptoms akin to Li, but its use remains limited[13].

Li stabilizes mood and modulates neurotransmitter release through intracellular signalling pathways, including inositol monophosphate and glycogen synthase kinase-3β (GSK-3β), and enhances neurotrophic factor expression (e.g., BDNF). While its neurogenic effects are modest and insufficient to fully reverse neuronal atrophy in BD and DD [14,15]. Other treatments, such as valproate, benzodiazepines, and antipsychotic drugs, primarily regulate neurotransmitters but do not address neuronal structural deficits [16]. Novel therapies targeting neurogenesis and neurite repair are under investigation, including neurotrophin mimetics and cytokine-based therapies [17,18].

Magnesium (Mg), involved in neurotransmitter regulation, synaptic plasticity, and neuroprotection, is emerging as a potential adjunctive therapy for BD and DD. Mg stabilizes glutamate and GABA levels, reduces oxidative stress, and minimizes neuronal inflammation[19]. Studies have shown low Mg levels correlate with increased depressive symptoms in BD patients, and supplementation enhances mood stabilizers' efficacy [20,21]. It has been observed that Mg supplementation could be as effective as lithium in treating rapidly cycling bipolar patients[22].

As Li is also considered to reduce the frequency and severity of manic and depressive episodes in BD, while Mg supplementation helps stabilize mood by improving neurotransmission, combining Li with Mg in the treatment of bipolar disorder (BD) is now generating considerable interest due to their complementary mechanisms, which may enhance therapeutic outcomes [13,23]

Currently, Li and Mg are usually administered orally in solid dosage forms such as tablets which often results in suboptimal bioavailability, potentially limiting their therapeutic efficacy. Factors such as gastrointestinal pH and interactions with food can affect the absorption, leading to very low serum concentrations and necessitating careful monitoring to avoid toxicity. Secondly, the oral administration also leads to higher absorption of minerals when the tablet is disintegrated, leading to much higher serum concentrations for a very short period which may be toxic, followed by low pharmacologically inactive blood levels throughout the day [24]. Similarly, solid forms, especially those containing poorly soluble salts like oxides, sulphates, or chlorides, exhibit limited absorption due to competition with other ions in the gastrointestinal tract. Liquid preparations also contain these salts which are poorly absorbed.

In contrast, liquid formulations, particularly those containing Li^+ and Mg^{2+} in ionic forms, may offer enhanced bioavailability. The immediate availability of ions in these solutions may facilitate more efficient absorption in the small intestine, potentially leading to more consistent therapeutic effects [25]. This improved absorption could allow for lower dosing, reducing the risk of side effects associated with higher doses of tablet forms and probably long-lasting therapeutically active serum levels throughout the treatment period.

Hydroxydase is the most Li^+-rich water in the world (8-9 mg/L), equally containing as high as 243 mg/L Mg^{2+}. Being a perfectly stabilized natural association of Li^+ and Mg^{2+} in an ionized bioavailable form, our aim was to evaluate whether synergistic effects of these two elements could help grow neurons and neurites, a prerequisite for the treatment of BD and DD. To better quantify the effects of Hydroxydase, we used *in vitro* neuronal cell cultures [27] and compared the efficacy against Li-carbonate, the most common salt used in solid tablets.

The objectives were to check whether a naturally stabilized, bioavailable, and ionized form of Li^+ and Mg^{2+} which can be easily and rapidly absorbed and used by the neuronal cells, could help neuronal cell growth as well as stimulate the growth and the length of the neurites, the basic requirements for an effective treatment of BD /DDs.

Materials and Methods

Test products: Cell culture medium containing 1% serum was used for cell controls and for test product dilutions. All test products were added in the culture medium at a volume not exceeding 5% total culture medium volume. Li-carbonate stock concentrations were prepared by dissolving 2.0 mg Li-carbonate salt in 10 ml (200 µg/ml) culture medium. The concentration used for experiment assumed that for a human of 70 kg, 600 to1200 mg of Li-carbonate is usually administered as daily dose, equivalent to 8 to 16 mg/kg (8-16 µg/g). These doses maintain a blood concentration of about 22-45 Mg/L (0.6 – 1.2 mEq/L or 22 to 45 µg/ml) [27,28]. For *in vitro* testing, we prepared culture mediums containing 20 µg/ml (1%), 60 µg/ml (3%) and 100µg/ml (5%) representing mean to double concentrations of Li carbonate to which neuronal cells are likely to be exposed during Li therapy.

Hydroxydase water containing 9737µg/L Li^+ and 243 mg/L Mg^{2+} was used in 200 ml, no air contact filled glass bottles. Hydroxydase water was used unchanged at 1%, 3%, and 5% concentrations in the culture medium. Each experiment was performed in triplicate and repeated at least twice. Mean values of minimum 6 tests were then calculated[29].

Cell cultures: Commercially available cryopreserved rat cortical neurons were purchased (A10008041, Invitrogen) and were cultured as per the suppliers' recommendations using a slightly modified method as described by Facci et al [30]. In short, cells were quickly thawed by gently spinning in a 37°C water bath for about 1–2 minutes and then transferred to a 50 mL sterile tube. 20 mL of warmed culture medium (neurobasal medium, containing 2mM L-glutamine, antibiotics and 4% foetal bovine serum), was added to the cell-containing tube. 100 µl of medium containing 10^3 cells /ml density was introduced in each of the 12-wells of culture plate (Corning) coated with poly-D-lysine and laminin. Cells were then incubated in a 37° C humidified incubator with 5% CO_2 for at least 1h to allow cell attachment. The culture medium was then replaced by 1ml culture medium containing 1% serum and desired concentrations (1%, 3%, or 5%) of the test products in the culture medium. Each experiment was repeated at least thrice.

Image analyses: Cell cultures were imaged after 1, 3, and 7 days of culture as described by M. Pool et al [26]. On days 1 and 3, images of each plate were taken with an inverted microscope, making sure to keep the settings constant between images. The culture dishes were returned to the incubation chamber until final image analyses and fixation on day 7.

Neuronal cell count and neurite growth measurements: During the initial step, we used standard microscope image analyses to determine cell number, neurite branches originating for the cells, and neurite length as described by Rønn et al [31]. The mean number of neuronal cells was

quantified by counting total number of neurons in a fixed area of the photo for all the 3 images per dilution at each time point. The total neurite length per cell was estimated by counting the number of intersections between neurites using conventional computer-assisted microscopy to measure the length. The absolute length of neurites per cell was subsequently estimated from the number of neurite intersections per cell.

These data obtained through conventional image tracing method were further confirmed through freely available Fiji ImageJ package, including the NeuronJ plugin for neurite tracer analysis [32]. The plugin analyses fluorescence microscopy image processor measures number of cells / area, the neurite outgrowths, and the neurite length of each neuron visible in the defined area of cell culture image. The results are presented as mean number of cells, number of neurites, and length of neurites (µm) in minimum 2 cultures (n=6). The length of neurite(s) in each cell was measured for all the cells present in the observation field, added, and mean value per cell (µm) was determined.

Statistical analysis: Student t test was used to compare the mean and ± SD of the mean between negative cell cultures treated with culture medium and Hydroxydase or lithium carbonate treated groups. One way ANOVA was used to compare the differences between controls and Hydroxydase.

Results

On day 7, in Hydroxydase-treated cell cultures, a total of 22 cells are visible while other cells are not attached to the surface. The cells have neurites but not clearly visible. After NeuronJ processing, the neurite growth of each cell is clearly visible and can be measured.

The images in Figure 1 and 2 are provided as an example to illustrate the technique used as well as the parameters measured in this study. The images in **Figure. 1** and **Figure. 2** are from Madeline pool et al. [26].

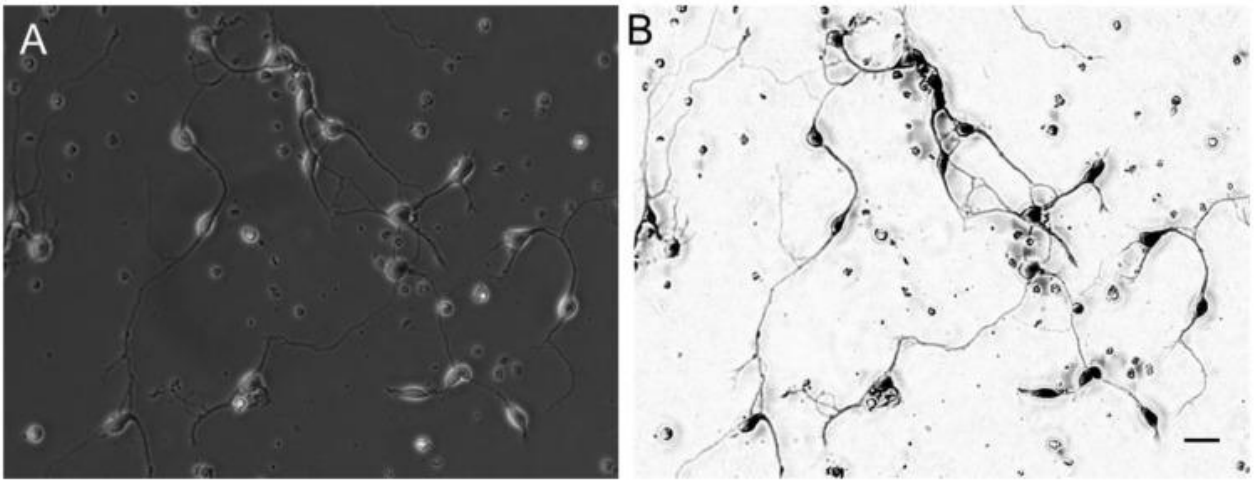
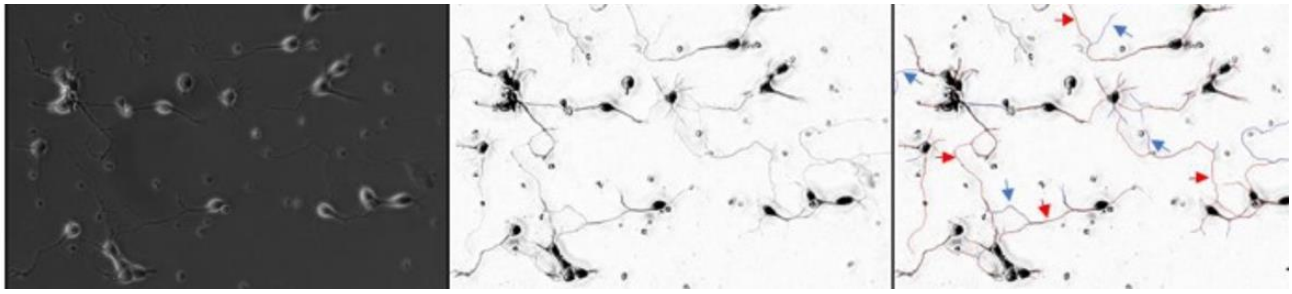


Figure 1: *Phase contrast image before (A) and after (B) preparation in ImageJ. The neurites in (B) are much more visible and clearer to calculate the number of cells and neurite growth. Bar = 50µm.*



All the images in **Figure. 2** represent the same cell culture. The 1st image (**Figure. 2-A**) represents neuron cell culture observed under the phase contrast microscope, the same culture in image analysis (**Figure. 2-B**) and under neurite tracer (**Figure. 2-C**). The colour of the trace in Fig. 2-C indicates whether the neurites are originated from the soma (primary neurite, tracing in red arrows), or a secondary neurite (tracing in blue arrows).

All neurites, whether primary or secondary, are considered as individual neurons and the total length of all the neurites originating from a neuron was measured. The data obtained by counting neurite intersections correlated statistically with results obtained using conventional microscopic image tracing and through Neurite Tracer, a neurite tracing plugin program.

Neuron observations on days 1, 3, and 7 for the three test product concentrations: The results are shown in **Table 1.**

Concentration exposed	**Day after treatment**	**Mean N° of cells (n=3)**	**Mean N° of neurites/cell (n=3)**	**Mean length of neurites µm (n=3)**
Group 1: Culture medium %				
1.0%	Day 1	14,2	12.32 ± 0.21	169.25 ± 18.36
	Day 3	16,6	14.51 ± 0.41	145.65 ± 23.40
	Day 7	16,1	12.55 ± 0.55	139.52 ± 31.64
3.0	Day 1	15,3	13.26 ± 0.60	178.15 ± 22.80
	Day 3	16,6	14.35 ± 0.74	156.28 ± 14.32
	Day 7	15,4	16.41 ± 0.29	155.20 ± 27.70
5.0%	Day 1	17,0	13.92 ± 0.15	183.08 ± 32.05
	Day 3	15,9	15.28 ± 0.55	174.08 ± 30.07
	Day 7	16,2	16.15 ± 0.48	174.38 ± 19.37
Mean		**15.92** ± *0.85*	**14.30** ± *1.46*	**163.95** ± *15.30*
Group 2: Lithium carbonate salt solution 20 µg/ml				
1.0%	Day 1	13,6	13.44 ± 0.53	134.15 ± 31.34
	Day 3	15,5	14.10 ± 0.65	181.30 ± 18.31
	Day 7	17,8	12.10 ± 0.37	157.39 ± 30.48
3.0%	Day 1	16,2	14.15 ± 0.28	156.38 ± 34.39
	Day 3	17,7	13.95 ± 0.48	192.88 ± 27.28
	Day 7	15,6	13.15 ± 0.82	175.66 ± 36.85
5.0%	Day 1	16,7	15.11 ± 0.74	169.32 ± 41.25
	Day 3	17,3	14.26 ± 0.41	203.47 ± 29.18
	Day 7	16,0	15.68 ± 0.35	184.36 ± 25.66
Mean		**16.26** ± *1.31*	**13.99** ± *1.04*	**172.76** ± *21.12*
Group 3 : Hydroxydase water				
1.0%	Day 1	14.4	15.32 ± 0.62	142.99 ± 64.6
	Day 3	17.5	16.30 ± 1.21	241.44 ± 72.00
	Day 7	18.1	18.31 ± 0.87	279.50 ± 65.27
3.0%	Day 1	15.6	15.68 ± 0.71	188.77 ± 59.34
	Day 3	20.9	19.14 ± 0.84	231.88 ± 94.44
	Day 7	18.6	23.35 ± 0.45	346.63 ± 96.74
5.0%	Day 1	16.1	16.36 ± 0.64	169.45 ± 84.31
	Day 3	20.1	21.10 ± 0.59	264.87 ± 105.81
	Day 7	22.4	26.40 ± 1.26	375.08 ± 61.29
Mean		**18.19** ± *2.61*	**19.10** ± *3.829*	**248.96** ± *77.58*

Table 1*: Effect of exposure of three different concentrations of either culture medium, Li-carbonate salt solution 20µg/ml or Hydroxydase Li^{+} + Mg^{2+} rich water on rat hippocampal neurons in vitro. The observations were made after 24h (Day 1), and on days 3 and 7 on the growth of neuron cells, as well as on the mean number of neurites and the mean neurite length (µm) per cell. The values represent observations in 3 cultures for total number of cells per well, the mean number of neurite outgrowth in the cells of a culture well and the total length of neurites in these cells (µm) ± SD of the means.*

The results from this study highlight several important observations regarding the effects of Hydroxydase water compared to the culture medium and Li-carbonate salt solution 20µg/ml on neuronal growth parameters.

Cell culture controls: At the start of the study, the neuron cultures were nearly identical with respect to the number of neuronal cells, number of neurites per cell, and the neurite length. This indicates that the experimental conditions began with comparable baselines, ensuring the

observed effects are attributed to the treatments. On day 0, when the cells were cultured, there were no neurites. This reinforces that all neurite growth observed during the study resulted from the treatments applied during the experimental period.

Effect on mean number of neuron cells: The mean number of cells with three different concentrations, in control cultures exposed only to the culture medium was 15.92±0.85 and 16.26±1.31 in the Li-carbonate control group, showing no significant difference between these two groups (**Figure. 3**). Cells treated with 1%, 3% or 5% Hydroxydase water, the number of cells was slightly higher compared to culture medium exposed controls and Li-carbonate group. While the difference is not statistically significant (18.19±2.61), it reflects the potential of Hydroxydase to support cell proliferation more effectively as well as absence of cytotoxic effects at all the test concentrations for the three test products.

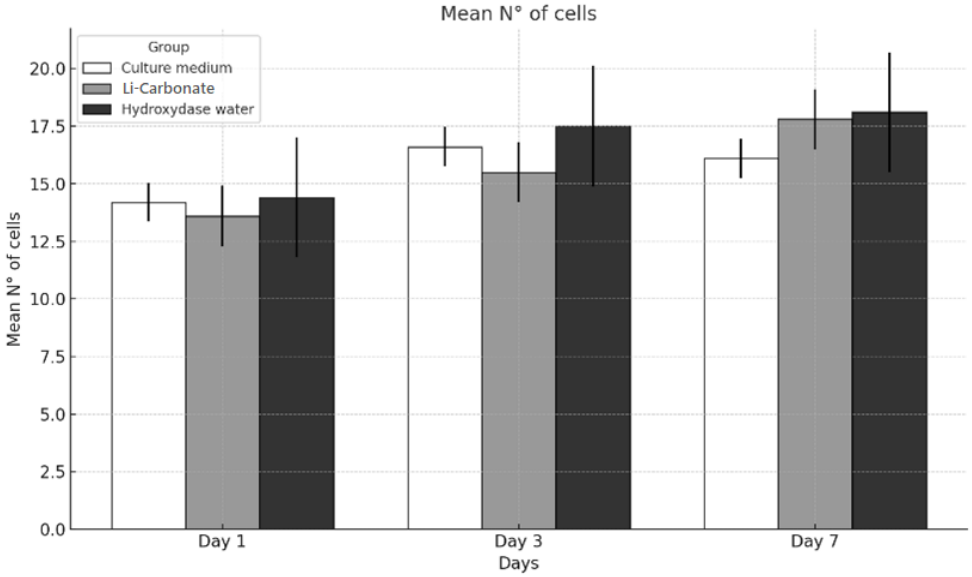


Figure 3: *Effect on mean number of neuronal cells in cell cultures exposed to 1%, 3%, and 5% concentrations of culture medium (white bars), Li-carbonate solution (grey bars) and to Hydroxydase water (black bars). The bars represent mean values observed on days 1, 3, and 7 for the three test product concentrations for each test liquid ± SD.*

Number of neurites per Cell: Although Hydroxydase water had little effect on the growth of the number of cells compared to the controls, its effect on neurite formation was much more pronounced (**Figure. 4**). In the Hydroxydase group, the mean number of neurites was nearly up to 30% higher (19.10±3.8) compared to the culture medium (14.30±1.46) and the Li-carbonate group (13.99±1.04) This demonstrates that Hydroxydase significantly stimulates the generation of new neurites, indicating a strong effect on neuronal differentiation.

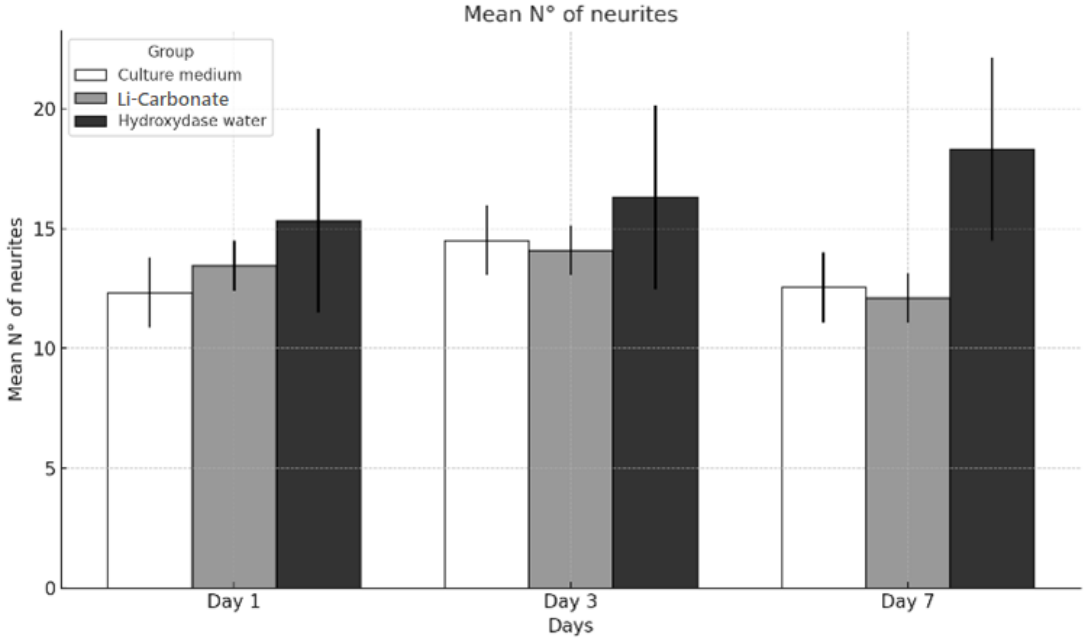


Figure 4*: Effect on the mean number of neurites on day 1, 3, and 7 exposed to culture medium (white bars), Li-carbonate solution (grey bars) and to Hydroxydase water (black bars) ± SD of the mean. The bars represent mean values observed on days 1, 3, and 7 for the three test product concentrations for each test liquid ± SD.*

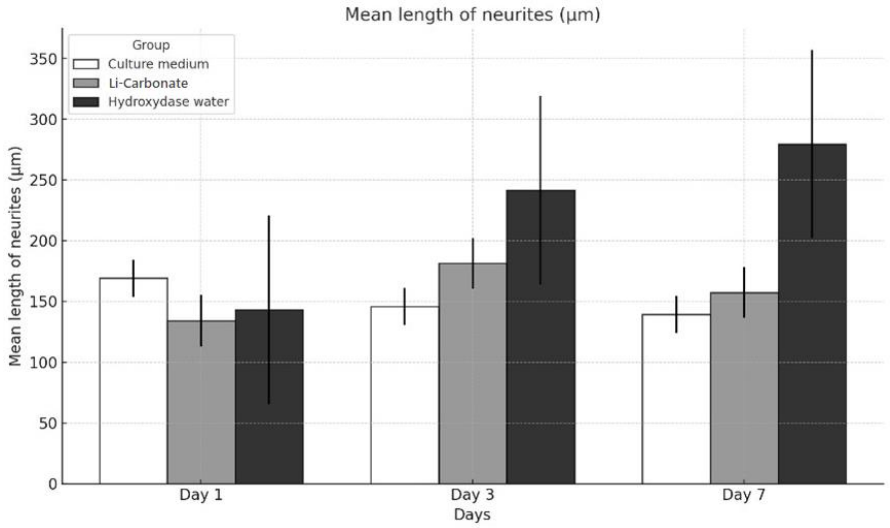


Figure 5*: Effect on the length of neurites in µm on day 1, 3, and 7, exposed to culture medium (white bars), to Li-carbonate solution (grey bars) and to Hydroxydase water (black bars) ± SD of the mean. The bars represent mean values observed on day 1, 3, and 7 for the three test product concentrations for each test liquid ± SD.*

Effect on the mean length of neurites: Neurite growth started on Day 1 in all groups and progressed steadily up to Day 7 with all treatments showing gradual increases in neurite length. However, the mean growth of neurites was significantly faster in the Hydroxydase group (248.96±77.58). By the end of the study on day 7, the neurite length in the Hydroxydase group was nearly 47% higher compared to both the culture medium (163.95±15.30) and Li-carbonate group (172.76±21.12). This suggests that Hydroxydase water not only stimulates neurite generation but also promotes rapid neurite elongation.

The findings from this study clearly demonstrate that while Hydroxydase water has a modest effect on cell proliferation, it remarkably improves the growth and elongation of neurites within a short period of time. This effect is observed across all three concentrations of Hydroxydase water. The increased number and length of neurites highlight Hydroxydase water's potential as a powerful enhancer of neuronal differentiation and connectivity which should promote neurotransmission *in vivo*.

Discussion

Neuronal degeneration is a significant feature observed in mood disorders such as BD and DD. This degeneration is characterized by reductions in neuron density, neurite numbers, and neurite length [5]. Studies have demonstrated poor neurite density in neuron cultures treated with serum from patients with bipolar disorder, particularly in the late stages of the illness [33]. This neuronal loss minimizes synaptic connections and impairs the transmission of electrical signals, leading to weakened neuronal networks that regulate mood and behaviour [2]. Slower and less efficient transmission of neuronal action potentials further exacerbates functional impairments in brain circuits involved in emotional regulation and stress response. These impairments contribute to mood instability, memory deficits, impaired decision-making, and episodes of mania or depression [3,34]. Additional contributors include mitochondrial dysfunction, reduced levels of brain-derived neurotrophic factor (BDNF), and overactivation of glutamate receptors such as N-methyl-D-aspartate (NMDA). The combination of these neurodegenerative factors leads to BD and DD [35,36]. Therefore, only treatments that reverse neurodegeneration and stimulate neuronal growth can effectively minimize the symptoms of BD and DD.

Current treatments for BD and DD primarily address symptoms without focusing on repairing or regenerating neurons [37]. Antidepressants and mood stabilizers, such as selective serotonin reuptake inhibitors (SSRIs) and anti-inflammatory drugs, provide symptomatic relief but fail to reverse neuronal damage. Neurotrophic therapies like lithium Li aim to enhance BDNF levels, promoting neuronal growth and repair; however, their efficacy remains limited. Furthermore, Li salts cause multiple side effects, including tremors, weight gain, and organ toxicity, necessitating regular blood level monitoring [38]. The use of Mg, either alone or in combination with Li, is also underexplored.

The results of this study demonstrate that exposure of neuronal cells to Li bicarbonate alone is non-cytotoxic at concentrations as high as 100 µg/ml, with no significant effects on neurite generation or length. The median daily doses of Li carbonate or other Li salts range between 600 and 1200 mg/day, administered twice or thrice daily, generating blood concentrations between 0.6 and 1.2 mEq/L (22–44 mg/L). These serum concentrations are too low compared to the doses administered [27]. Although Li is a powerful mood-stabilizing medication, its exact mechanism of action remains unclear, and it has a narrow therapeutic range due to poor intestinal absorption [39]. Thus, finding a safer alternative form of Li, particularly for long-term treatment, is essential.

Hydroxydase, a natural mineral water from the volcanic Auvergne region in France, is rich in ionized lithium (Li^+ 9740 µg/L) and magnesium (Mg^{2+} 243 mg/L). It has been used since 1923 for improving physical and emotional well-being [40] and as a cellular function enhancer [41]. While the exact mode of action underlying its mood-stabilizing properties is unknown, the neuroprotective and neurite growth-promoting effects observed in this study may explain its efficacy. These effects were not seen with Li alone, suggesting that the richness of Hydroxydase in ionized and bioavailable Li and Mg and their synergistic effects on brain neurons are responsible for these properties. Mg levels are known to be low in patients treated with Li-salts [42]. Compared to synthetic Li salts, Li^+ in Hydroxydase is likely to better stabilize neurotransmitter levels, promote neuronal growth, and repair structural brain alterations associated with mood disorders. Mg complements Li by acting as an NMDA receptor antagonist, reducing neuronal excitation, modulating neurotransmitter release, and protecting neurons from oxidative damage and inflammation. The exact synergistic mode of action of Li and Mg on neuronal growth still remains unexplored but may have a protective effect on neuronal degeneration through multiple pathways including stimulation of anti-aging gene functions. For instance, it has already been demonstrated that

anti-aging gene Sirtuin 1 is critical to neuron proliferation, differentiation and survival, where Mg and Li may play a role of activator, minimizing neuronal degeneration and occurrence of BD/DD [43,44].

The balanced composition of Li and Mg in Hydroxydase is likely to enhance their combined efficacy, providing consistent therapeutic effects without the peaks and troughs associated with tablet-based treatments containing Li salts, such as Li carbonate [45]. Delivered in small, measured doses, Hydroxydase ensures uniform absorption and avoids the cytotoxicity often linked to synthetic drugs. It is bottled directly at the source to preserve its ionized minerals, ensuring long-term stability and effectiveness.

Despite its therapeutic potential, Hydroxydase remains underutilized due to the absence of clinical trials examining the synergistic effects of Li and Mg. As a natural product, it cannot be patented, making it less attractive to pharmaceutical companies. Nevertheless, its unique composition and historical efficacy position it as a valuable alternative for managing BD and DD.

Limitations of the study: This study was conducted using *in vitro* neuronal cultures, a controlled environment that excludes neurohumoral influences present *in vivo*. As such, the observed synergistic effects of Li and Mg on neuronal growth may not fully translate to *in vivo* conditions. Furthermore, the test substance, Hydroxydase water, contains not only high concentrations of Li and Mg, but also various other minerals and trace elements, which may have contributed to the observed effects. Therefore, caution must be exercised when extrapolating these *in vitro* findings to potential therapeutic outcome.

Conclusion

Further research is needed to validate and explore the therapeutic benefits of ionized and bioavailable lithium (Li) and magnesium (Mg) as a synergistic, safer, and natural alternative to conventional therapies for treating BD and DD. By addressing the underlying causes of neuronal degeneration, $Li^{+}Mg$ treatments hold promise for more effective management of BD and DD.

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