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# Histone Deacetylase Inhibition Ameliorates Oxidative Stress in Alzheimer's Disease

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**ABSTRACT**

**Aim:** Alzheimer's disease (AD), is linked to oxidative stress, which accelerates neuronal damage and cognitive decline. Sodium butyrate, has antioxidant properties and capacity to control gene expression, We aimed to study the potential therapeutic effect of the histone deacetylase (HDAC) inhibitor sodium butyrate alone or its combination with vitamin E (Vit.E) on oxidative stress biomarkers in an experimental model of (AD) induced by $AlCl_3$ in the rat. **Methods:** Rats injected with $AlCl_3$ subcutaneously (10 mg/kg) daily for 8 weeks. From the 5th week of $AlCl_3$injection afterwards, rats were administrated sodium butyrate (50,100 or 200 mg/kg), Vit.E (25 mg/kg), sodium butyrate (100 mg/kg) + Vit. E (25 mg/kg), and donepezil (10 mg/kg) orally along with $AlCl_3$. Lipid peroxidation (malondialdehyde: MDA), reduced glutathione (GSH), and nitric oxide (NO) were measured in brain homoigenates. **Results:** compared with the saline-treated control group, rats given $AlCl_3$ showed significant and marked increases in brain NO and MDA levels, Meanwhile, GSH showed significant decrease when compared with the saline control group. The results of those parameters in $AlCl_3$- injected rats treated with sodium butyrate showed significant improvement, also vit.E and donepezil were effective to reduce MDA and NO. **Conclusions:** the results show that sodium butyrate has neuroprotective, and anti-oxidative roles which guide us to a new treatment for AD disease.

## Introduction:

Alzheimer's disease (AD) is a neurodegenerative progressive disorder that mainly affects older adults being the most cause and lead to dementia in the world. It is usually accompanied with memory impairment, cognitive decline,

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and behavioral changes **[1].** The accumulation of amyloid-beta plaques, extensive neuronal loss, and tau tangles are the most pathological marks of AD **[2].**

The amyloid-beta peptide, is known to stimulate production of reactive oxygen species [ROS], and causes oxidative damage of neurons. It can cause disruption to the cellular functions where it may cause mitochondrial dysfunction, which further magnifies oxidative stress **[3].** Where mitochondria considered as the primary affected sites of ROS generation, the mitochondrial dysfunction results due to the excessive production of ROS **[4].**

Sodium butyrate is short-chain fatty acid produced during the fermentation of dietary fiber in the gut, have drawn interest as a neuroprotective agent. It has been found that sodium butyrate have several biological effects. Its ability in regulating oxidative stress and its possible therapeutic applications for Alzheimer's disease are examined in this research **[5].**

Sodium butyrate has the ability to inhibit histone deacetylase (HDAC) which is the enzyme that control the gene expression by changing the histone protein. As the HDACs are inhibitedby the effect of sodium butyrate, the expression of the gene that promote neuronal survival increase. Sodium butyrate also improves mitochondrial function, and reduces inflammation **[6].** Some studies have proposed that sodium butyrate may reduce amyloid-beta accumulation in the brain by encouraging the clearance of amyloid plaques or avoiding their creation **[7].**

Another pathogenic characteristic of AD is hyperphosphorylated tau proteins, which facilitate microtubule instability and neurofibrillary tangle development, which is linked to elevated oxidative stress and neuronal degeneration **[8].**

Antioxidants aid in the neutralization of ROS and shield cells from oxidative stress. Antioxidants like vitamin E, vitamin C, and polyphenols may lessen oxidative damage and delay the onset of AD, according to studies **[9].** Clinical investigations, however, have yielded conflicting findings, and research on their efficacy is still underway

## Material and Methods:

### Drugs and chemicals

Donepezil, hydrochloride from (Pfizer Egypt, Cairo, A.R.E.), Aluminum chloride from (Sigma, USA), and sodium butyrate from (Sigma-Aldrich, USA), were dissolved in distilled water, vitamin-E from (Pharco Pharmaceuticals, Alexandria, Egypt) was dissolved in olive oil. All drugs were freshly prepared immediately before use.

### Animals

(80) Adult male Sprague-Dawley rats, there weigh 170-180 g, were brought from the Animal House Colony of Agriculture faculty–Alexandria University. All used animals were housed under the conventional laboratory conditions during the experimentation period at room temperature of 25± 2 °C, 60 to 70 % humidity, 12 hour light/dark cycle, fed standard laboratory pellets (5% fats, 1% multivitamins, 20% proteins), and legalized free access to tap water. After one week of accommodation, rats were group-housed into 8 groups (each group had 10 rats), and all experiments adhered to the ethical considerations in handling laboratory animals of the Ethics committee of the Science Faculty, Fayoum University. The local committee approved the design of the experiments with (2025-AEC2331-b).

### Experimental design:

The following groups were studied:

**Group 1** was treated with saline and act as negative control.

**Group 2** was injected with $AlCl_3$ (10 mg/kg) subcutaneously each day **[10].**

**Group 3** injected with $AlCl_3$ (10 mg/kg) subcutaneously + sodium butyrate 50 mg /kg orally**[11,13]**.

**Group 4** injected with $AlCl_3$ (10 mg/kg) subcutaneously + sodium butyrate 100 mg /kg orally **[12,13]**.
**Group 5** injected with $AlCl_3$ (10 mg/kg) subcutaneously + sodium butyrate 200 mg /kg orally**[13]**.
**Group 6** injected with $AlCl_3$ (10 mg/kg) subcutaneously + Vit.E (25 mg/kg ) orally daily **[14]**.
**Group 7** injected with $AlCl_3$ (10 mg/kg) subcutaneously + Vit.E (25 mg/kg) + sodium butyrate (100mg/kg) orally daily.
**Group 8** injected with $AlCl_3$ (10 mg/kg) subcutaneously + donepezil (10 mg/kg) orally daily**[15].**
$AlCl_3$ injected for 8 weeks every day subcutaneously and from the beginning of $5^{th}$ week of the study the used drugs administrated orally for 4 weeks. Finally, animals were sacrificed under the effect of anesthesia after 24 hour from the latest treatment. Brains were quickly removed then washed by ice-cold saline solution (0.9% NaCl), and stored at -80°C until assayed for biochemical parameters. They were homogenized with 0.1 M phosphate-buffered saline (pH 7.4) to obtain 10% homogenate for biochemical measurements.

**Biochemical assays**

**lipid peroxidation**

Lipid peroxidation was assessed by measuring the level of malondialdehyde (MDA) in brain tissues. Malondialdehyde was determined by measuring reactive species of thiobarbituric acid, where thiobarbituric acid-reactive substances react with thiobarbituric acid and produce red-colored complex having a peak-absorbance 532 nm. The produced pink color was measured by using ultraviolet (UV)-VI8 recording spectrophotometer **(Shimadzu Corporation, Rydalmere, Australia)** with wavelength of 532 nm in contrast to the blank solution, which was prepared by adding 0.25 mL of distilled water to 2.25mL of the working reagent **[16].**

**ELISA measurements**

Brain levels of reduced glutathione (GSH) and nitric oxide (NO), were determined by ELISA using commercially available kits from Sun-Long Biotech Co., LTD (China) according to the manufacturer's protocol

**Statistical analysis**

The recorded data were represented as mean ± SD. one way analysis of variance (ANOVA), was used to analyze The data and Tukey's test for multiple comparisons was applied. Graph pad prism (version 6) software was used for analyzing data. The results with the probability of $p \leq 0.05$ were considered statistically significant

**Results**

**1.Lipid peroxidation**

Results presented in table (1) showed: significant increase in brain lipid peroxidation (MDA) level in $AlCl_3$ (control group) compared to the saline control group (21.2± 1.1*vs.* 10.3± 0.9 nmol/g tissue, $p \leq 0.05$). Sodium butyrate administration resulted in dose-dependent and significant decrements in MDA in $AlCl_3$ injected rats as following (13.7± 0.47, 12.9 ± 0.3, 10.7 ± 0.5 vs. $AlCl_3$ control value 21.2± 1.1 nmol/g. tissue, $p \leq 0.05$). A significant decrease in MDA also were observed after treatment with vitamin.E or vitamin.E+ sodium butyrate 100 mg/kg (12.8 ± 0.4 and 10.6 ± 1.2 vs. $AlCl_3$ control value 21.2± 1.1 nmol/g. tissue, $p \leq 0.05$). Meanwhile, MDA decreased by treatment with donepezil (14.1 ± 1.1 vs. $AlCl_3$control value 21.2± 1.1nmol/g. tissue, $p \leq 0.05$).

**2. Nitric oxide**

Results presented in table (2) showed that compared with the saline control group, $AlCl_3$-injected rats exhibited significantly higher nitric oxide level (11.1 ± 0.7 vs. saline control group 7.6 ± 0.7 µmol/L , $p \leq 0.05$ ). In the groups treated with sodium butyrate, the nitric oxide level fall compared with the $AlCl_3$ control value (9.2 ± 1.1, 8.7 ± 1 , 7.7 ± 0.7 vs. 11.1 ± 0.7 µmol/L, $p \leq 0.05$).

Meanwhile, rats given Vit.E or Vit.E + sodium butyrate 100 mg/kg showed decrease in nitric oxide (8.8 ± 0.7 , 8.1 ±1 vs. $AlCl_3$ control value 11.1 ± 0.7µmol/L). On the other hand, rats treated with donepezil showed decrease in brain nitric oxide as (9.1 ± 1.8 vs. $AlCl_3$ control value 11.1 ± 0.7µmol/L, $p \leq 0.05$).

**3- Reduced glutathione**

Results presented in table (3) showed significant decrease in rats brain content of their GSH in the $AlCl_3$-injected rats related to the saline control (362.8 ± 26.4 vs. 522 ± 47ng/L, $p \leq 0.05$). In groups that received sodium butyrate with doses of : 50, 100 and 200 mg/kg, a significant increase in GSH level was observed, respectively compared with the $AlCl_3$ control group as following (438 ± 26.34, 441.6 ± 47.9, and 464.8 ± 23.5 vs. 362.8 ± 47 ng/L , $p \leq 0.05$ ). In $AlCl_3$ +Vit.E or $AlCl_3$ + Vit.E + sodium butyrate , GSH levels increased compared by the $AlCl_3$ control group ( 447.1 ± 31.7 and 456.5± 28.4 vs. 362.8 ± 26.4 ng/L , $p \leq 0.05$ ). On the other hand, rats treated with donepezil showed increase in brain GSH compared with $AlCl_3$ control group as (442.7 ± 41.3 vs. 362.8 ± 26.4 ng/L , $p \leq 0.05$).

## Discussion.

AD is a neurodegenerative disease marked by extracellular amyloid plaques, intraneuronal neurofibrillary tangles, neuropil formation, oxidative stress, and synaptic loss. Numerous theories, including the oxidative stress, tau, amyloid, cholinergic, and neuro-inflammatory theories, try to explain the pathophysiology of AD **[17].**

In order to comprehend AD pathophysiology at the molecular, cellular, and behavioral levels and to create novel treatment medicines, it is crucial to use appropriate animal models. Aluminum has effects similar to those of cholinotoxins on neuronal structure, as well as blood-brain barrier (BBB) permeability **[18].** Exposure to aluminum chloride is known to modify the BBB, impact axonal transports, induce inflammatory responses, alter synapse structure, and result in significant memory loss **[19].** Prolonged exposure to $Al^{+3}$ causes neurologic signs that resemble progressive neuro-degeneration in the cerebral cortex, hippocampus, and spinal cord, confirming the possible neurotoxicity effect of aluminum in experimental animal models as described in previous studies **[20,21**].

The administration of $AlCl_3$ to rats in this study increased oxidative stress in the brain by increasing lipid peroxidation and nitric oxide, it also cause a decrease in the antioxidant reduced glutathione. These findings are consistent with another published research that detected elevated brain lipid peroxidation and decreased glutathione in the brains of rats and mice injected with $AlCl_3$. [**22, 23, 24].**Since oxidative damage displaces iron from its binding sites, it increases the availability of metal transition to take part in redox reactions that harm cells, which was thought to be a key mechanism of the neurotoxicity induced by $Al^{+3}$ [**25**, **26**].In this work, sodium butyrate and vitamin E were found to increase reduced glutathione and decrease the rise in brain malondialdehyde in the brains of $AlCl_3$injected rats. This shows that there neuroprotective effects were aided by an antioxidant process. Additionally, we discovered a significant rise in the amount of nitric oxide in the rats' brains after they were given AlCl**3**, which is corroborated by other research. [27, 28**].**

Reactive nitrogen oxides that can undergo nitration, oxidation, and nitrosylation processes, as well as peroxynitrite (ONOO-), are responsible

for the neurotoxic consequences of high nitric oxide concentrations **[29, 26].** In the meantime, it has been noted that blocking nitric oxide synthases provides neuro-protection in the brains of rats injected with $AlCl_3$, indicating a significant role for nitric oxide in Al-induced neurotoxicity **[30].** According to this study, when sodium butyrate mixed with vitamin E and administered to rats that had received an $AlCl_3$ injection, there was a notable drop in brain nitric oxide level. This result suggests that the neuro-protective action of sodium butyrate and vitamin E may entail nitric oxide suppression**.**

**Conclusion**: In this experimental model of AD produced with repeated $AlC_3$ injection to rats, sodium butyrate has proved of therapeutic value inhibiting oxidative stress which is main step in neurodegeneration in AD.

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**Table (1) MDA level (nmol/g tissue) in Rat brain tissue of the different groups:**

| | Mean± Std. Deviation | % of change from control | % of change from $AlCl_3$ | $p^{(a)}$ value | $P^{(b)}$ value |
|---|---|---|---|---|---|
| Control (group 1) | 10.3± 0.9 | | | | |
| $AlCl_3$ (group 2) | 21.2± 1.1 | ↑106.2% | | < 0.0001 | |
| $AlCl_3$+Sod 50 (group 3) | 13.7± 0.5 | ↑33.8% | ↓35.1% | < 0.0001 | < 0.0001 |
| $AlCl_3$+Sod 100 (group 4) | 12.9± 0.3 | ↑55.2% | ↓39.3% | < 0.0001 | < 0.0001 |
| $AlCl_3$+Sod 200 (group 5) | 10.7± 0.5 | ↑3.8% | ↓49.7% | 0.9879 | < 0.0001 |
| $AlCl_3$+Vit.E (group 6 ) | 12.8± 0.4 | ↑25.1% | ↓39.4% | <0.0001 | < 0.0001 |
| $AlCl_3$+Vit.E + Sod 100 (group 7) | 10.6± 1.1 | ↑3.2% | ↓50% | 0.9957 | < 0.0001 |
| $AlCl_3$+donepezil (group 8) | 14.1± 1.1 | ↑37.5% | ↓33.3% | < 0.0001 | < 0.0001 |

$P^{(a)}$ value versus control group
$P > 0.05$ non-significant value
$P < 0.001$ highly significant

$P^{(b)}$ value versus $AlCl_3$ group
$p \leq 0.05$ significant

**Table (2): Rat Brain Nitric Oxide (NO) level u mol/L in the different groups:**

| | Mean± Std. Deviation | % of change from control | % of change from $AlCl_3$ | $P^{(a)}$ value | $P^{(b)}$ value |
|---|---|---|---|---|---|
| Control (group 1) | 7.6± 0.7 | | | | |
| $AlCl_3$ (group 2) | 11.1± 0.7 | ↑45.6% | | < 0.0001 | |
| $AlCl_{3+}$Sod 50 (group 3) | 9.2± 1 | ↑20.1% | ↓17.5% | 0.1839 | 0.0369 |
| $AlCl_{3+}$Sod 100 (group 4) | 8.7± 1 | ↑14% | ↓21.7% | 0.6108 | 0.0044 |
| $AlCl_{3+}$Sod 200 (group 5) | 7.7± 0.7 | ↑1.2% | ↓30.5% | > 0.9999 | 0.0001 |
| $AlCl_{3+}$Vit.E (group 6 ) | 8.8± 0.7 | ↑14.5% | ↓21.13% | 0.5667 | 0.0053 |
| $AlCl_{3+}$Vit.E + Sod 100 (group 7) | 8.1± 1 | ↑5.2% | ↓27.73% | 0.9973 | 0.0001 |
| $AlCl_3$+donepezil (group 8) | 9.1± 1.8 | ↑19.1% | ↓18.2% | 0.2333 | 0.0268 |

$P^{(a)}$ value versus control group

$P$ >0.05 non-significant value

$P$ <0.001 highly significant value

$P^{(b)}$ value versus $AlCl_3$ group

$p \leq 0.05$ significant value

**Table (3)Rat Brain Reduced glutathione (GSH) level** (pg/ml) **in different groups:**

| | Mean± Std. Deviation | % of change from control | % of change from $AlCl_3$ | $P^{(a)}$value | $P^{(b)}$ value |
|---|---|---|---|---|---|
| Control (group 1) | 522± 47 | | | | |
| $AlCl_3$ (group 2) | 362.8± 26.4 | ↓30.5% | | < 0.0001 | |
| $AlCl_{3+}$Sod 50 (group 3) | 438± 26.3 | ↓16.2% | ↑20.7% | 0.0042 | 0.0140 |
| $AlCl_{3+}$Sod 100 (group 4) | 441.6± 47.9 | ↓15.5% | ↑21.7% | 0.0069 | 0.0086 |
| $AlCl_{3+}$Sod 200 (group 5) | 464.8± 23.5 | ↓10.96% | ↑28.1% | 0.1216 | 0.0003 |
| $AlCl_{3+}$Vit.E (group 6 ) | 447.1± 31.7 | ↓14.3% | ↑23.2% | 0.0146 | 0.0040 |
| $AlCl_{3+}$Vit.E + Sod 100 (group 7) | 465.5± 28.4 | ↓10.8% | ↑28.3% | 0.1305 | 0.0003 |
| $AlCl_3$+donepezil (group 8) | 442.7± 41.3 | ↓15.3% | ↑22% | 0.0081 | 0.0074 |

$P^{(a)}$ value versus control group
$P$ >0.05 non-significant value
$P$ <0.001 highly significant value

$P^{(b)}$ value versus $AlCl_3$ group
$p \leq 0.05$ significant value