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The Neuroprotective and Hepatoprotective Effects of the Histone Deacetylase Inhibitor Sodium Butyrate Against Ketamine-Induced Acute Neuronal and Liver Injury

Amany A. Sleem [1*], Omar M. E. Abdel-Salam [2], Eman R. Youness [3] and Enayat A. Omara [4]

[1] Pharmacology Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, 12622, Egypt.
[2] Toxicology and Narcotics Department, Medical Research and Clinical Studies, Institute National Research Centre, Cairo, 12622, Egypt.
[3] Medical Biochemistry Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, 12622, Egypt.
[4] Pathology Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, 12622, Egypt.

In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
The effects of the histone deacetylase inhibitor sodium butyrate were evaluated in ketamine-induced neurotoxicity and liver injury in the rat. Ketamine was intraperitoneally (i.p.) administered in a single dose of 35 mg/kg, and rats were treated at the same time with either saline or sodium butyrate at 100 or 200 mg/kg. Rats were euthanized 4h later. Biochemical markers of oxidative stress: malondialdehyde, reduced glutathione, nitric oxide as well as paraoxonase 1 activity were estimated in the brain and liver. In addition, brain amyloid beta (Aβ)-peptide and acetylcholinesterase (AChE) concentrations were determined. Histological examination of brain and liver sections was also performed. A significant increase in malondialdehyde and a significant decrease in reduced glutathione and paraoxonase 1 were found in the brain and liver after injection of ketamine, whereas a significant decrease in nitric oxide was observed in the brain tissue. Moreover, ketamine-treated rats exhibited significantly lower levels of Aβ-peptide and AChE compared to the saline control. Sodium butyrate treatment significantly reduced malondialdehyde levels, and increased both reduced glutathione, and paraoxonase 1 in brain and liver, but had no significant effects on nitric oxide levels. Furthermore, sodium butyrate treatment caused further decrease in Aβ-peptide concentrations and restored AChE concentrations in brain compared to ketamine controls. Ketamine induced diffuse degeneration in cerebral cortex and severely degenerated hepatocytes. Sodium butyrate resulted in marked alleviation of the histologic damages. These results suggest the potential use of sodium butyrate in the treatment of neurotoxicity associated with ketamine abuse and possibly in other neurodegenerative states.
Keywords: ketamine; sodium butyrate; histone acetylation; neurotoxicity; hepatotoxicity

1. Introduction

Ketamine is commonly used analgesic drug for induction and maintenance of analgesia in emergency medicine. It is administered intravenously at low subanesthetic doses for pain control or inducing mild sedation [1]. It is also used as an adjuvant drug to general anesthesia to reduce postoperative pain and opioid requirements [2,3] and in combination with morphine for control of moderate to severe acute pain in humans [4]. The drug is a phenylcyclidine hydrochloride derivative, which binds noncompetitively to the phencyclidine receptors binding site of *N*-methyl-D-aspartate (NMDA) glutamate receptor. Ketamine is a dissociative anesthetic, with psychoactive properties [5]. The drug is rapidly distributed in the central nervous system and at subanaesthetic doses, produces unusual dissociative effects with distortions of space and time awareness, hallucinations as well as depersonalisation [6,7]. With very high doses of ketamine, users experience and enjoy a state of profound detachment from reality, the so called 'K-hole' [7]. In addition to enhanced sexual, musical, and sensory delight, ketamine users also experience paranoid ideation [8]. Due to its hallucinogenic effects, ketamine is a common drug of abuse, particularly by young people during raves, big music festivals, and clubs [9,10].

*Corresponding author e-mail: omasalam@hotmail.com.
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Neurotoxic effects following high doses of ketamine were observed in developing animals and in certain brain regions of adult rats without cerebral injury [11]. Magnetic resonance imaging studies revealed structural brain changes in adolescent-onset and adult-onset users of ketamine [12]. *In vitro*, exposure of forebrain neurons from newborn rats to ketamine caused oxidative protein damage, and neurotoxicity, indicative of increase in reactive oxygen species production [13]. In adult rats, single i.p. injection of ketamine, induced dark shrunken neurons, neuronal apoptosis, pericellular vacuolations, and increased caspase-3 immunoreactivity in brain [14,15]. The neurotoxic effects of ketamine are caused by increase in oxidative stress and were amenable to treatment with antioxidants such as L-carnitine [13], *N*-acetylcysteine [15] or methylene blue [16]. Oxidative stress is considered an important mechanism that underlies neuronal cell death in a number of neurodegenerative and neurological disorders [17]. Oxidative stress develops when the rate of formation of reactive oxygen species and other free radicals, exceeds the capacity of the antioxidant mechanisms of the cell. This results in oxidative damage to the cell membranes, by inducing lipid peroxidation, enzyme proteins, and nucleic acids, ultimately leading to perturbation of cellular functions and even cell death [18].

Histone acetylation, determined by the balance between histone acetyltransferases and histone deacetylases, regulates gene expression. Histone acetyltransferases increase protein histone acetylation, resulting in the release of condensed chromatin, and stimulates transcription and gene expression. In contrast, histone deacetylases catalyze deacetylation of histone proteins at lysine residues and promote chromatin condensation, thereby, inhibiting transcription and gene expression. Therefore, treatment with histone deacetylase inhibitors, promote posttranslational acetylation of lysine residues within nuclear and cytoplasmic proteins [19,20]. Sodium butyrate is a short-chain fatty acid, produced through anaerobic bacterial fermentation of dietary fibers in the lower intestinal tract [20,21]. Sodium butyrate acts as a histone deacetylase inhibitor, which regulates gene expression in the brain, and has been shown to exert neuroprotective effects [22,23].

In this study, therefore, we aimed to investigate the effects of sodium butyrate on oxidative stress, neurotoxicity and hepatic injury induced by the dissociative anesthetic ketamine in the rat.

2. Materials and methods

2.1. Animals

Male adult Sprague-Dawley strain rats weighing between 170 and 180 g were used in this study. Rats were housed in uniform settings with unrestricted access to water and a standard lab diet. The guidelines set forth by the Institute Ethics Committee and the U.S. National Institutes of Health's Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996) were followed when conducting the animal studies.

2.2. Chemicals and reagents

Sigma (St Louis, MO, USA) provided the sodium butyrate. The Ministry of Justice in Egypt provided ketamine. Sigma (St. Louis, USA) provided additional chemicals and reagents. An isotonic (0.9% NaCl) saline solution was used to dissolve the sodium butyrate and ketamine prior to usage. The residual chemicals and reagents were obtained from Sigma (St Louis, MO, USA) and were of analytical grade. Based on earlier research, the dosages of sodium butyrate and ketamine used were selected [16,24,25].

2.3. Experimental groups

The following groups (6 rats per group) were used: saline (0.2 ml/rat, i.p.) was given to group 1 (the normal control). Ketamine at 35 mg/kg was administered intraperitoneally (i.p.) to groups 2, 3, and 4. Group 2 was then maintained as a positive ketamine control and given i.p. saline. In the meantime, 100 or 200 mg/kg of sodium butyrate were administered to groups 3 and 4. After four hours, the rats were decapitated and put under a light ether anesthetic. Their brains and livers were promptly removed, and they were immediately cleaned with an ice-cold 0.9% NaCl solution, weighed, and stored at -80°C. For the biochemical tests, the tissues were homogenized with 0.1 M phosphate buffer saline at pH 7.4, resulting in a final concentration of 10% w/v. Representative liver and brain samples were preserved in 10% neutral buffered formalin for the histopathological investigations.

2.4. Biochemical analyses

2.4.1. Oxidative stress

Malondialdehyde, reduced glutathione, and nitric oxide were measured in brain and liver homogenates to identify biomarkers of oxidative stress. Using the technique developed by Nair and Turne [26], the end product of lipid peroxidation, malondialdehyde, was measured by thiobarbituric reactive substances (TBAS). The TBA-MDA adduct is created when TBAS and thiobarbituric acid react, and a spectrophotometer is used to measure the absorbance at 532 nm. Using Ellman's reagent [DTNB (5,5'-dithiobis (2-nitrobenzoic acid)], which is reduced by the free sulfhydryl group on the GSH molecule to produce 5-thio-2-nitrobenzoic acid, reduced glutathione (GSH) was ascertained. The latter's color is yellow, and its absorbance at 412 nm can be measured with a spectrophotometer [27]. Using Griess reagent, the amount of nitrate/nitrite, or nitric

oxide, was estimated. In this assay, nitrate reductase transforms nitrate into nitrite. After that, the nitrite and Griess reagent combine to generate a deep purple azo compound. With a spectrophotometer set to 540 nm, the absorbance is measured [28].

2.4.2. Paraoxonase-1 activity

Phenyl acetate was utilized as a substrate in a colorimetric technique to ascertain the arylesterase activity of paraoxonase 1. In this experiment, phenol is produced through the cleavage of phenyl acetate catalyzed by paraoxonase 1. By tracking the rise in absorbance at 270 nm at 25°C, the rate of phenol formation was determined. One µmole of phenol formed per minute is equivalent to one unit of arylesterase activity. Based on the phenol extinction coefficient of 1310 $M^{-1}cm^{-1}$, the paraoxonase 1 activity is expressed in kU/l [29].

2.5. Histological studies

A light microscope (Olympus Cx 41 with DP12 Olympous digital camera. Olympous optical Co. Ltd, Tokyo, Japan) was used to examine slides prepared from five µm thick paraffin sections that had been stained with haematoxlin and eosin [30].

2.6. Statistical analyses

The study's data were shown as mean ± SEM. Duncan's multiple range test was used in conjunction with a one-way ANOVA to determine statistical significance. The program used was Graphpad Prism version 6 (GraphPad Prism Software Inc., San Diego, CA, USA). A probability value was deemed statistically significant if it was less than 0.05.

3. Results

3.1. Brain parameters

3.1.1. Brain oxidative stress

The MDA level in the brain of rats given i.p. injection of ketamine was significantly higher than that of the saline controls (46.6% increase: 32.87 ± 0.75 *vs.* 22.42 ± 1.39 nmol/g.tissue). Meanwhile, reduced glutathione (GSH) was significantly reduced by 27% by ketamine treatment (2.46 ± 0.07 *vs.* 3.37 ± 0.1 µmol/g.tissue). In addition, ketamine-treated rats showed significant decrease in brain nitric oxide by 15.8%, compared to their saline controls (15.08 ± 0.39 *vs.* 17.91 ± 0.43 µmol/g.tissue) (Fig. 1).

In ketamine-treated rats, sodium butyrate given at 100 or 200 mg/kg resulted in significant decrease of the elevated MDA level by 19.1% and 36% in comparison to the ketamine control (26.58 ± 1.25 and 21.03 ± 0.77 *vs.* 32.87 ± 0.75 nmol/g tissue). A significant increase in GSH by 33% was observed after the highest dose of sodium butyrate (3.28 ± 0.06 *vs.* 2.46 ± 0.07 µmol/g.tissue). However, administration of sodium butyrate to rats treated with ketamine had no significant effect on the levels of nitric oxide (Fig. 1).

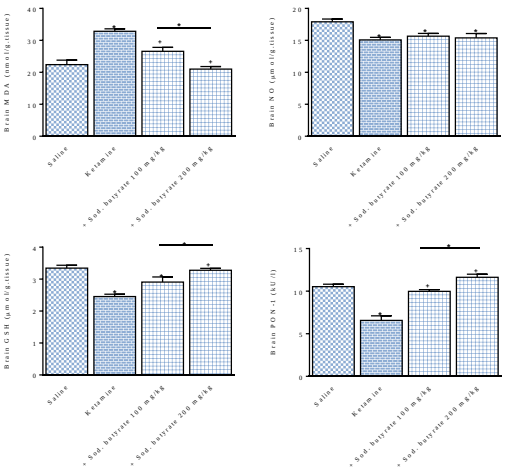

Figure 1. The effects of sodium butyrate (Sod.butyrate) on oxidative stress parameters (nitric oxide, reduced glutathione, and malondialdehyde) and paraoxonase-1 in brains of rats given i.p. ketamine. *p<0.05 *vs*. the saline control and between the different groups as shown in the graph. +p<0.05 *vs*. ketamine control.

3.1.2. Brain paraoxonase-1

The activity of paraoxonase 1 in the brain after 4h of ketamine injection showed significant decrease by 37.6% compared to the saline group (6.57 ± 0.54 *vs.*10.53 ± 0.28 kU/l). This decrease in paraoxonase 1 activity by ketamine was prevented by the administration of sodium butyrate at 100 mg/kg and 200 mg/kg of sodium butyrate (9.98 ± 0.2 and 11.63 ± 0.36 *vs.* 6.57 ± 0.54 kU/l) (Fig. 1).

3.1.3. Brain amyloid Aβ-peptide

In rats receiving i.p. inection of ketamine, the concentration of amyloid Aβ-peptide was significantly decreased by 59.6% compared to the saline control (4.32 ± 0.34 vs.10.68 ± 0.19 pg/ml). Amyloid Aβ-peptide showed further decrease by 54.4% and 52.3% after the administration of sodium butyrate at 100 or 200 mg/kg, respectively, compared to the ketamine control (1.97 ± 0.22 and 2.06 ± 0.14 vs. 4.32 ± 0.34 pg/ml) (Fig. 2).

3.1.4. Brain acetylcholinesterase

The i.p. administration of ketamine at 35 mg/kg resulted in significant decrease in the brain AChE concentration by 55.8% compared to saline control (1.49 ± 0.08 *vs.* 3.37 ± 0.17 ng/ml). The reduction in AChE by ketamine was prevented by the administration of sodium butyrate at 100 or 200 mg/kg (3.11 ± 0.07 and 3.41 ± 0.06 *vs.*1.49 ± 0.08 ng/ml) (Fig.3).

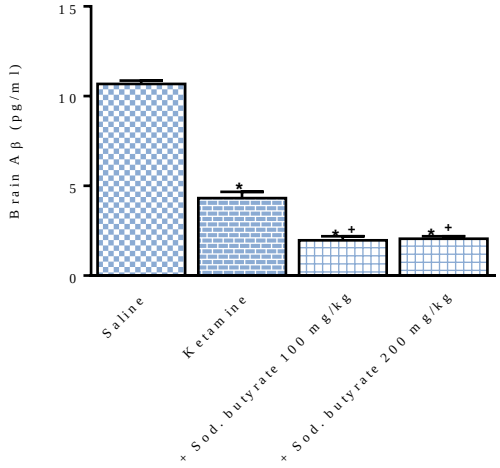

Figure 2. Effect of sodium butyrate (Sod.butyrate) on levels of amyloid Aβ peptide (Aβ) in brain of rats treated with i.p. ketamine. *p<0.05 *vs.* saline control. +p<0.05 *vs.* ketamine control.

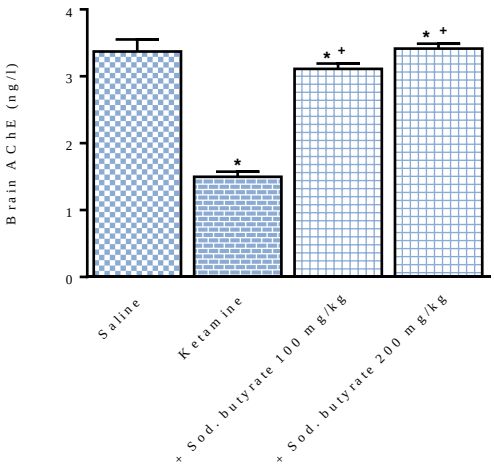

Figure 3. Effect of sodium butyrate (Sod.butyrate) on levels of acetylcholinesterase (AChE) in brain of rats given i.p. ketamine. *p<0.05 *vs.* saline control. +p<0.05 *vs.* ketamine control.

3.2. Liver parameters

3.2.1. Liver oxidative stress

Liver MDA in rats given ketamine was significantly higher by 67.7% compared to the saline control (57.2 ± 1.26 *vs.* 34.1 ± 1.33 nmol/g.tissue). Moreover, the GSH level in the liver tissues of ketamine-treated rats, was significantly lower by 31.6%, than in the saline control group (2.95 ± 0.13 *vs.* 4.31 ± 0.12 µmol/g.tissue). No significant effect on liver nitric oxide, was observed in ketamine-treated animals in comparison to their saline controls (26.56 ± 0.40 *vs.*28.27 ± 1.0 µmol/g.tissue) (Fig. 4).

In ketamine-treated rats receiving sodium butyrate, there were significant decreases in MDA levels by 27.4% and 37.7% compared to ketamine control (41.51 ± 4.32 and 35.64 ± 2.54 *vs.* 57.2 ± 1.26 nmol/g tissue). Liver GSH in the ketamine treated group was significantly increased by 200 mg/kg sodium butyrate (28.5% increase: 3.79 ± 0.26 *vs.* 2.95 ± 0.13 µmol/g.tissue). The level nitric oxide, however, was not significantly changed in rats administered sodium butyrate compared to the ketamine control group (23.77 ± 0.64 and 23.0 ± 0.37 *vs.* 26.56 ± 0.40 µmol/g tissue) (Fig. 4).

3.2.3. Liver paraoxonase-1

Liver paraoxonase-1 activity was significantly lower in ketamine-treated rats by 49.8% compared with the saline control group (15.61 ± 0.71 *vs.* 31.11 ± 0.94 kU/l). The activity of paraoxonase-1 in liver of ketamine-treated animals increased by 24.1% after the administration of 100 mg/kg of sodium butyrate (18.61 ± 0.49 *vs.* 15.71 ± 0.61 kU/l). Rats receiving 200 mg/kg of sodium butyrate exhibited paraoxonase-1 activity close to the saline control value (28.63 ± 1.14 *vs.* 31.11 ± 0.94 kU/l) (Fig. 4).

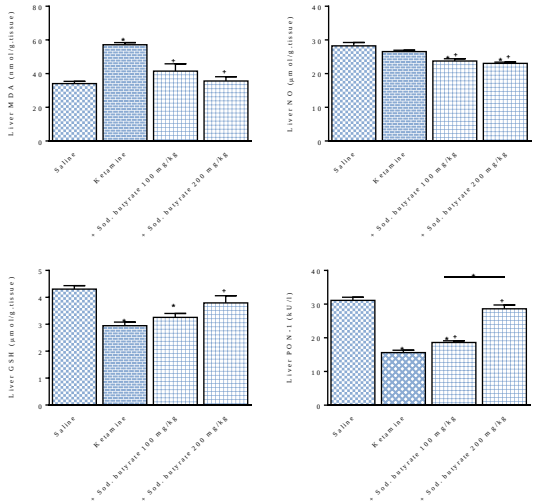

Figure 4. Effect of sodium butyrate (Sod.butyrate) on liver oxidative stress parameters (malondialdehyde, nitric oxide, reduced glutathione) and paraoxonase-1 in rats given i.p. ketamine. *p<0.05 *vs.* saline control and between the different groups as shown in the graph. +p<0.05 *vs.* ketamine control.

3.3. Effect of sodium butyrate on ketamine–induced histologic damage

3.3.1. Brain histologic damage

Sections of the cerebral cortex of saline control rats, showed normal histological structure of the meninges, and neurons of the cerebral cortex. Neuro-glial cells had well demarcated lightly stained nuclei (Fig. 5A). Ketamine caused diffuse degeneration in the cerebral cortex, with severe dilatation and congestion in meningeal blood vessels. The cerebral cortex showed congestion of cerebral blood vessels, dark shrunken cortical neurons, that had apoptotic cells, deeply stained pyknotic nuclei with pericellular

vacuolations. Glial cells appeared normal with lightly or darkly stained nuclei (Fig. 5B). Sections from the ketamine and sodium butyrate 100 mg/kg-treated group showed moderate ameliorative effect, with mild pericellular vacuolations and mild dilated and congested blood vessels. Apoptotic cells and pyknotic nuclei were also observed. Glial cells appeared moderately normal with lightly or darkly stained nuclei (Fig.5C). Rats treated with ketamine and sodium butyrate 200 mg/kg showed minimum damage as demonstrated by reduced neuronal cell damage in the cerebral cortex and preservation of the normal cells, with mild dilated blood vessels. Glial cells appeared nearly normal, that had lightly or darkly stained nuclei. Some apoptotic cells and pyknotic nuclei were also observed (Fig. 5D).

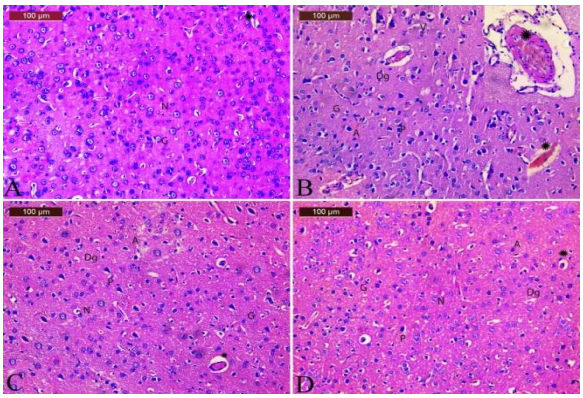

Figure 5. Representative photomicrographs of brain sections (Hx & E) after treatment with: **(A)** Saline showing normal cortex neurons having vesicular nuclei (N), and basophilic cytoplasm, blood vessels with narrow perivascular spaces (Bv) and glial cells (G); **(B)** Ketamine showing diffuse degeneration in the cerebral cortex, with severe dilatation and congestion in meningeal blood vessels, apoptotic cells (Ap), deeply stained pyknotic nuclei (P) with pericellular vacuolations (V), congestion of cerebral blood vessels (BV). Glial cells were normal having lightly or darkly stained nuclei (Dg); **(C)** Ketamine and sodium butyrate 100 mg/kg showing moderate ameliorative effect with mild pericellular vacuolations (V), mild dilated and congested blood vessels (Bv), apoptotic cells (Ap) and pyknotic nuclei (P), Glial cells were moderately normal in appearance with lightly (G) or darkly stained nuclei (Dg); **(D)** Ketamine and sodium butyrate 200 mg/kg showing minimum damage to the brain tissue, as demonstrated by the reduced neuronal cell damage and preservation of normal cells with mild dilated blood vessels (Bv). Glial cells exhibited nearly normal appearance with lightly (G) or darkly stained nuclei (Dg), minimal apoptotic cells (Ap) and pyknotic nuclei (P).

3.3.2. Liver histologic damage

Sections of liver tissue from the saline control group showed normal hepatic structure, classic hepatic lobules, each formed of hepatocytes arranged in cords, radiating from the central veins to the periphery of the lobules. The cell cords were separated by blood sinusoids.

They had eosinophilic cytoplasm and central large vesicular nuclei with prominent nucleoli (Fig. 6A).The animals treated with ketamine showed that hepatic lobules had lost their normal hepatic architecture, with dilatation and congestion of the central veins and haemolysis of blood cells in blood sinusoids. Some areas showed dispersed severe degenerated hepatocytes, enlarged and ballooned hepatocytes. Patchy areas of highly eosinophilic cells appeared distorted with deeply stained nuclei.

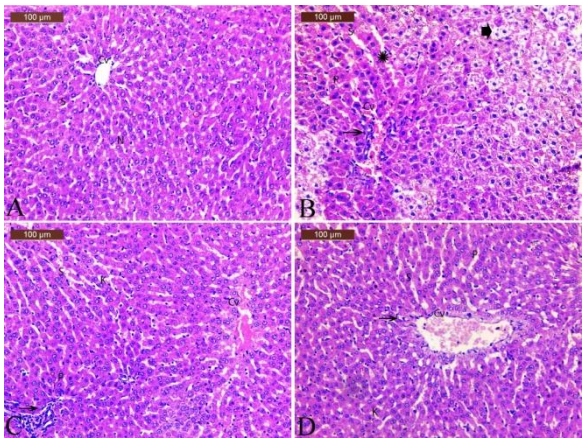

Figure 6. Representative photomicrographs of Hx & E stained liver sections after treatment with: **(A)** Saline showing cords of normal hepatocytes radiating from the central vein (CV) and separated by blood sinusoids (S). The hepatocytes contained eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli (N); **(B)** Ketamine showing hepatic lobules that lost their normal hepatic architecture, together with dilatation and congestion of both the central veins (CV) and congestion and haemolysis of blood cells in blood sinusoids (S). Some areas showed dispersed severe degenerated hepatocytes, enlarged and ballooned, patchy areas of highly eosinophilic cells that appeared distorted with deeply stained pyknotic nuclei (P), focal areas of inflammatory cells infiltrations aggregation around central vein (arrow); **(C)** Ketamine and sodium butyrate 100 mg/kg showing moderate improvement indicated by the normal appearance of histological architecture, with normal hepatocytes with vesicular nucleus, except congestion central vein (CV) and sinusoids (S) with focally dispersed inflammatory cells aggregation in some areas (arrow), activated Kupffer cells (K) with pyknotic nuclei (P); **(D)** Ketamine and sodium butyrate 200 mg/kg showing restored normal histological architecture with normal appearance of central vein (Cv), hepatic cells, and sinusoids (S), with occasionally seen mild inflammatory cells aggregation around central vein (arrow), activated Kupffer cells (K) with pyknotic nuclei (P).

Focal area of inflammatory cells infiltrations aggregation around central vein was observed (Fig. 6B). The administration of sodium butyrate at 100 mg/kg caused moderate improvement, evidenced by normal appearance of histological architecture, with normal hepatocytes having vesicular nuclei. However, congestion of central vein and sinusoids with focally dispersed aggregates of inflammatory cells in some areas, activated Kupffer cells with pyknotic nuclei were also noticed (Fig. 6C). Histological examination of hepatic sections from rats treated with sodium butyrate at 200 mg/kg, showed normal histological architecture, with normal appearance of central vein, hepatic cells, and sinusoids. Occasional mild inflammatory cells aggregation around central vein, activated Kupffer cells with pyknotic nuclei were also noticed (Fig. 6D).

4. Discussion

In the current study, we investigated the potential protective effect of the histone deacetylate inhibitor, sodium butyrate, in the brain and liver of ketamine-treated rats. Ketamine induced marked brain neuronal degeneration and hepatoceullar damage, associated with markedly raised oxidative stress levels. We found that treatment with sodium butyrate exerted antioxidant action, significantly lowering lipid peroxidation, and preventing the depletion of reduced glutathione and the decline in paraoxonase–1 activity caused by the anesthetic in both the rain and liver tissues. We also showed that treatment with sodium butyrate afforded histological protection against the ketamine-induced tissue injury.

The present work confirmed previous studies demonstrating degeneration and perineuronal vacuolations of cerebral cortex neurons as well as hepatocellular injury by ketamine [14,15,31]. We also demonstrated that ketamine given in a single i.p. dose of 35 mg/kg, resulted in significantly raised levels of oxidative stress, evidenced by the increase in the lipid peroxidation end product malondildehyde, which then, is indicative of an increased formation of reactive oxidative species and consequent oxidant attack on the cell membrane lipids [32]. This coincided with significant lowering of the levels of reduced glutathione in the brain and liver of ketamine-treated animals. The tripeptide glutathione (γ-glutamylcysteinylglycine) with its redox-active thiol group, is an important antioxidant which protects the cell against oxidative stress by direct scavenging of reactive oxygen/nitrogen species e.g., as hydroxyl radicals, lipid peroxyl radicals, and peroxynitrite, and electrophiles. It also acts indirectly being a co-factor for glutathione peroxidases. In presence of increased levels of oxygen/nitrogen species, reduced glutathione (GSH) is converted to its oxidized form GSSG [33,34]. The decrease in glutathione levels by ketamine, therefore, can be due to its consumption by an increase in reactive oxygen/nitrogen species and/or failure to replace the depleted antioxidant. The effect of ketamine on oxidative stress and cellular antioxidants are supported by other studies that demonstrated increased lipid and protein oxidation biomarkers and a decrease in levels of reduced glutathione, and in superoxide dismutase and catalase activities in rodent brain by ketamine [31,35,36].

We further demonstrated a significant inhibition of paraoxonase–1 activity following ketamine injection, which is in accordance with earlier studies [14,15,31]. Paraoxonase–1 is involved in xenobiotic metabolism, and in particular in hydrolyzing some of the organophosphorus insecticides [37]. The enzyme, which is synthesized by the liver and released into blood associated with high density lipoproteins, possesses an antioxidative and antiinflammatory effects. [38]. The decline in enzyme activity has been demonstrated in a number of neurological [39,40], and liver disorders [41]. Paraoxonase-1 is inactivated by oxidants [42], which then, render the cell exposed to oxidative events. Therefore, restoration of enzyme activity in brain and liver of ketamine-treated rats by the administration of sodium butyrate could be the result of lowered levels of oxidative stress, thereby, preventing inactivation of the enzyme. In addition, the preservation of liver architecture after sodium butyrate may be involved owing to the renewed ability of liver cells to synthesize the enzyme.

In this study and in accordance with previous observations [16], ketamine injection led to a significant decrease in brain AChE levels. Other researchers reported markedly increased acetylcholine release in rat brain after the injection of ketamine [43,44]. These effects of ketamine on cortical acetylcholine levels were suggested to be responsible for the neurotoxicity of NMDA antagonists [44] as well as for the hallucinogenic and psychotropic effects of the drug [6]. Our results showed that administering sodium butyrate was associated with restoration of AChE levels in brain of ketamine-treated animals. It may be suggested, therefore, that the decrease in AChE by ketamine reflects a neurotoxic action of the drug. Accordingly, the restoration of AChE levels by sodium butyrate confirms a neuroprotective effect of sodium butyrate.

We have also observed significant decrement in brain Aβ-peptide content after ketamine injection which is supported by previous studies [16]. The deposition of amyloid β-protein, the major component of amyloid plaques in the brain, is a pathological hallmark of Alzheimer's disease, the most common cause of late-onset dementia [45]. These Aβ deposites are considered the initiating event in the neuronal degeneration in Alzheimer's disease by promoting neuroinflammation and oxidative stress [46]. The mechanism that underlies the effect of ketamine on brain Aβ-peptide is not

clear. Interestingly, treatment with sodium butyrate was associated with further decline in Aβ-peptide levels, thereby, offering a new possibility for treating Alzheimer's disease.

Sodium butyrate, is a dietary produced, histone deacetylate inhibitor, which increases the transcription and expression of genes. Sodium butyrate is a metabolite gut microbiota produced through fermentation of dietary fibers. It is found in dairy milk and milk products e.g., butter and cheese [20]. In vivo studies demonstrated neuroprotective effects for sodium butyrate in experimental models of rat cerebral ischaemia [24,47], mouse spinal cord injury [23], neonatal hypoxia-ischemia [25], and transgenic Huntington's disease mice [22]. Neuroprotective effects were also reported in vitro, where sodium butyrate attenuated cell death in SH-SY5Y cells stimulated with TNF-α [48]. Sodium butyrate stimulated neurogenesis and up-regulated brain-derived neurotrophic factor protein levels in the ischaemic brain [47], inhibited the activation of the transcription factor Nuclear factor-kappa B [23], and reduced the expression of inflammatory cytokines e.g., interleukin 1β [24], inducible nitric oxide synthase and cyclooxygenase-2 as well as the levels of caspase-3 and caspase-1 [48]. The neuroprotective effects of sodium butyrate thus involve enhancing transcription and gene expression, thereby, activating a number of genes, and suppressing the inflammatory response.

5. Conclusions

In summary, this study showed that treatment with the histone deacetylate inhibitor sodium butyrate, protects the brain and liver tissue against the toxic actions of ketamine. Sodium butyrate ameliorated the increase in oxidative stress and decline in AChE and paraoxonase 1 activity induced by ketamine and afforded histological protection. Our results thus provided the first evidence that exogenously administered sodium butyrate is neuroprotective and hepatoprotective in ketamine-induced toxicity.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

This works was not supported by research grants.

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