

Brain Oxidative Stress and Neurodegeneration in the Ketamine Model of Schizophrenia during Antipsychotic Treatment: Effects of *N*-Acetylcysteine Treatment

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ABSTRACT | We aimed to investigate the effect of *N*-acetylcysteine (NAC) on brain oxidative stress and on the response to therapy with clozapine or haloperidol in the ketamine model of schizophrenia. Mice received intraperitoneal (i.p.) injections of either saline, ketamine (30 mg/kg), NAC (50 mg/kg), ketamine + NAC, ketamine + clozapine (1.5 mg/kg), ketamine + clozapine + NAC, ketamine + haloperidol (1.5 mg/kg), or ketamine + haloperidol + NAC daily for one week. Malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide, and paraoxonase-1 (PON-1) activity were determined in cerebral cortex and in the rest of the brain tissue. Results indicated that: (i) treatment with only NAC had no significant effects on MDA, GSH, or PON-1 activity, but resulted in a marked decrease in nitric oxide content in both the cortex and the rest of the brain compared to the saline control group; (ii) ketamine caused a significant increase in MDA, while decreasing nitric oxide, and GSH concentrations as well as PON-1 activity in both the cerebral cortex and the rest of the brain. These biochemical alterations were alleviated by NAC; (iii) in ketamine-treated mice, either clozapine or haloperidol had no significant effect on MDA or GSH levels. Nitric oxide, however, showed a significant increase by either agent. Meanwhile, PON-1 activity showed a significant increase by clozapine in the cortex but was decreased by haloperidol in both the cortex and the rest of the brain compared with the ketamine only group; (iv) NAC resulted in a significant decrease in MDA in the cortex in ketamine + haloperidol-treated mice, and markedly alleviated the decline in GSH and PON-1 activity in both the cortex and the rest of the brain tissue in ketamine + clozapine- or ketamine + haloperidol-treated groups. NAC showed variable effects on brain nitric oxide in these groups; and (v) ketamine caused neurodegeneration in the cortex and striatum changes in the form of shrunken neurons, pyknotic and apoptotic nuclei, perineuronal vacuolations, and red neurons. These pathological changes were marked after treatment with either clozapine or haloperidol but ameliorated by NAC treatment. These data indicate increased brain oxidative stress in the schizophrenia model induced by ketamine in mice and that treatment with antipsychotics impairs brain antioxidants. The study suggests a potential therapeutic benefit for NAC in alleviating brain oxidative stress, lipid peroxidation, and neurodegeneration during antipsychotic drug therapy in schizophrenia.

KEYWORDS | Clozapine; Haloperidol; Ketamine; Lipid peroxidation; *N*-Acetylcysteine; Neurodegeneration; Reduced glutathione; Schizophrenia

ABBREVIATIONS | GSH, reduced glutathione; MDA, malondialdehyde; NAC, *N*-acetylcysteine; NMDA, *N*-methyl-*D*-aspartate; PON-1, paraoxonase-1

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1. INTRODUCTION

Schizophrenia is a devastating mental disorder with detrimental effects on thought, speech, and affection, along with the development of hallucinations, delusions, and cognitive impairment [1, 2]. The manifestations of the disease are ascribed to increased subcortical release of dopamine and decreased activation of D1 receptors in the prefrontal cortex [3]. The brain in schizophrenic patients is subjected to high levels of free radicals, and a decreased level of reduced glutathione (GSH) has been found in the cerebral cortex, striatum, and cerebrospinal fluid of these patients [4–6]. There is evidence to suggest a deficit in glutathione synthesis in schizophrenia since decreased glutamylcysteine ligase modifier subunit gene expression was found in fibroblasts of schizophrenics [7]. Studies also indicated an increased level of nitric oxide in the brain of schizophrenic patients post-mortem [8] as well as increased lipid peroxidation and decreased activities of the antioxi-

dant enzymes superoxide dismutase and glutathione peroxidase in the plasma of chronic patients [9]. Moreover, increased urinary excretion of markers indicative of systemic oxidative damage to deoxyribonucleic and ribonucleic acids has been observed [10]. Ample evidence thus suggests an important role for oxidative stress in the pathogenesis and progression of schizophrenia [11–13]. In support of this notion are the recent studies demonstrating a benefit from adjunctive treatment with *N*-acetylcysteine (NAC) in schizophrenics. When given to these patients, NAC improved the deficit in auditory sensory processing [14] and resulted in greater improvement in symptoms [15, 16] including treatment-resistant patients [17]. NAC by providing the cysteine moiety necessary for GSH synthesis could increase GSH availability and correct the GSH deficit in the schizophrenic brain [18]. NAC has been shown to increase neuronal GSH and antioxidant capacity [19]. The GSH precursor NAC has thus become a promising therapeutic agent in psychiatric disorders, espe-

cially as an adjunctive therapy in cannabis use disorder in young people, negative symptoms in schizophrenia, and depression in bipolar disorder [20].

In the treatment of schizophrenia, antipsychotic drugs are used to block dopamine D2 receptors in the prefrontal cortex with the newer agents having in addition serotonin 5-HT_{2A} receptor blocking property. The latter is thought to account for the reduced ability of the newer generation antipsychotics to cause extrapyramidal manifestations [21]. Haloperidol and clozapine are two commonly used drugs for the treatment of schizophrenic symptoms. Haloperidol is a classic first-generation antipsychotic with predominantly dopamine D2 receptor blocking properties. Clozapine on the other hand belongs to the second generation antipsychotics having high 5-HT_{2A}/D2 affinity ratio, and demonstrated efficacy in treating resistant patients [22]. These drugs, however, have been shown to increase the generation of reactive oxygen metabolites and to cause oxidative stress in the plasma [23–26] and gray matter volume changes [27] in treated patients.

Schizophrenic symptoms could be induced in healthy humans by the administration of the dissociative anesthetics and glutamatergic *N*-methyl-*D*-aspartate (NMDA) receptor antagonists phencyclidine and ketamine [28, 29]. Thus, ketamine, a derivative phencyclidine hydrochloride is being widely used in rodents to induce a condition mimicking schizophrenia [30, 31]. In this study, the ketamine model of schizophrenia in mice was used to investigate the effect of NAC treatment on brain oxidative stress and on neurodegeneration during treatment with the classical antipsychotic haloperidol or the atypical antipsychotic clozapine.

2. MATERIALS AND METHODS

2.1. Animals

Swiss male albino mice, weighing 25–30 g, obtained from the Animal House of the National Research Centre (Cairo, Egypt) were used. Mice were group-housed under temperature- and light-controlled conditions and provided with standard laboratory food and water ad libitum. Animal use procedures were done in accordance with regulations of the Institutional Ethics Committee of the National Research Centre (Cairo, Egypt) and the recommendations of

the United States National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and Chemicals

N-Acetylcysteine (SEDICO Pharmaceutical Co., Cairo, Egypt), clozapine (Multi-Apex Pharma, Cairo, Egypt), haloperidol (Kahira Pharmaceuticals & Chemical Industries Co, Cairo, Egypt), and ketamine (Sigma–Aldrich, St. Louis, MO, USA) were used in the study. Ketamine and other drugs were dissolved in saline to obtain the necessary doses. Other chemicals and reagents were of analytical grade and purchased from Sigma–Aldrich.

2.3. Study Design

Mice were randomly allocated into eight equal groups, six mice each. Mice were treated with one of the following: saline (group 1), ketamine 30 mg/kg (group 2), NAC 50 mg/kg (group 3), ketamine + NAC (group 4), ketamine + clozapine 1.5 mg/kg (group 5), ketamine + clozapine + NAC (group 6), ketamine + haloperidol 1.5 mg/kg (group 7), ketamine + haloperidol + NAC (group 8). Drugs or saline was intraperitoneally (i.p.) injected daily for 7 days. Mice were euthanized 4 h after last injection and their brains were removed and dissected into cortex, and the rest of the brain tissue weighed and homogenized in 0.1 M phosphate buffer saline at pH 7.4 to give a final concentration of 10% (w/v) for the biochemical assays.

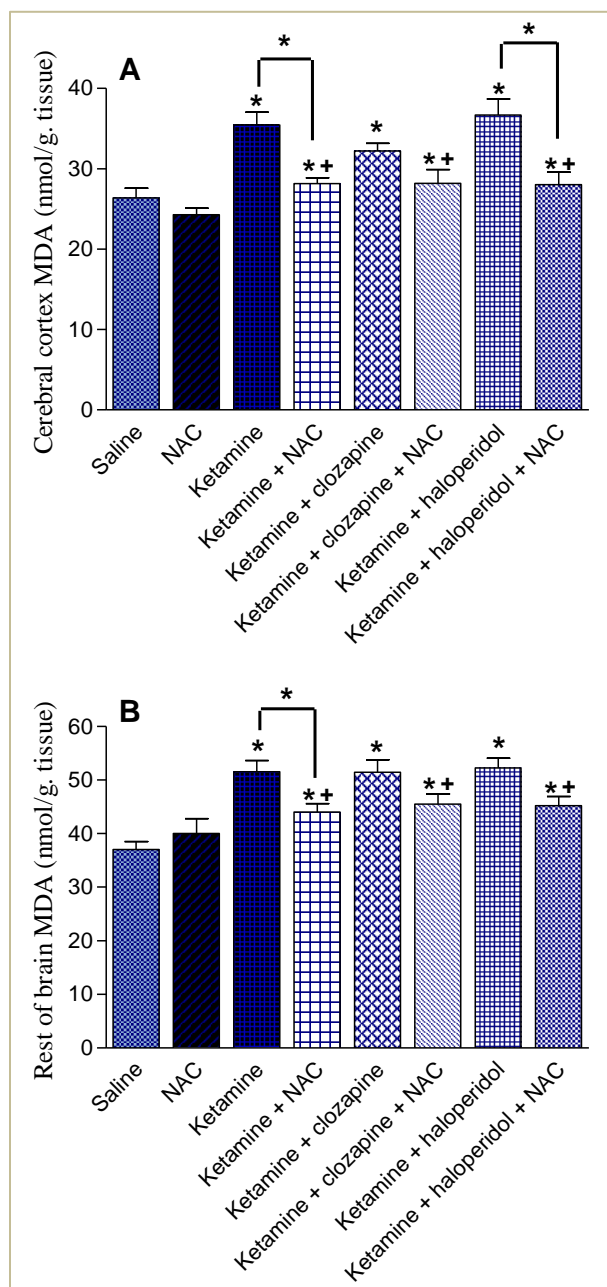
2.4. Biochemical Assays

2.4.1. Determination of Lipid Peroxidation

Lipid peroxidation was determined by measuring thiobarbituric acid reactive species (TBARS) according to Ruiz-Larrea et al. [32] and the produced red color substance was measured at 532 nm using a spectrophotometer (Shimadzu, Japan).

2.4.2. Determination of GSH

The content of GSH was determined according to the method of Ellman et al. [33]. DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] (Ellman's reagent) is reduced by the free SH group on GSH molecule and



the yellow colored 5-thio-2-nitrobenzoic acid produced is measured spectrophotometrically at 412 nm.

2.4.3. Determination of Nitric Oxide

Nitric oxide was determined using the Griess reagent. Nitrate is converted to nitrite via nitrate reductase. The Griess reagent then reacts with nitrite to form a

deep purple azo compound and the absorbance was read at 540 nm using a spectrophotometer [34].

2.4.4. Determination of Paraoxonase-1 (PON-1) Activity

PON-1 arylesterase activity in the brain homogenates was determined with the use of phenylacetate as a substrate. PON-1 catalyzes the cleavage of phenyl acetate with the formation of phenol. The rate at which phenol is produced is measured using a spectrophotometer by monitoring the increase in absorbance at 270 nm and 25°C. One unit of arylesterase activity is defined as 1 μmol of phenol formed per minute. Enzyme activity is calculated based on the extinction coefficient of phenol of $1310 \text{ M}^{-1}\text{cm}^{-1}$ at 270 nm, pH 8.0 and 25°C. Enzyme activity is expressed in kilo-international units/liter (kU/l) [35].

2.5. Histopathological Examination

The brain tissue sections were fixed in freshly prepared 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Paraffin sections of 5 μm thickness were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination under a light microscope.

FIGURE 1. Effects of *N*-acetylcysteine (NAC) administration on the levels of malondialdehyde (MDA) in the cerebral cortex (A) and the rest of the brain tissue (B) in mice treated with ketamine alone or with antipsychotic drugs. Data are means \pm SEM, evaluated by one-way analysis of variance and Duncan test. *, $p < 0.05$ vs. saline-treated group and between other groups as indicated in the figure; +, $p < 0.05$ vs. ketamine only.

2.6. Statistical Analysis

Data are presented as mean \pm SEM. The data are analyzed by one-way analysis of variance, followed by Duncan's multiple range test for post-hoc comparison of group means. Effects with a probability of $p < 0.05$ are considered statistically significant.

3. RESULTS

3.1. Oxidative Stress

3.1.1. Malondialdehyde (MDA) Levels

In normal mice, the administration of NAC at 50 mg/kg had no significant effect on MDA levels in the cerebral cortex and the rest of the brain tissue compared with saline-treated group (24.27 ± 0.83 and 40.0 ± 2.83 vs. control values of 26.38 ± 1.19 and 37.0 ± 1.53 nmol/g.tissue). Mice treated with ketamine at 30 mg/kg showed 34.3% and 39.3% increments in brain MDA levels in the cerebral cortex and the rest of the brain compared to the saline group, respectively (35.44 ± 1.62 and 51.0 ± 2.1 vs. control values of 26.38 ± 1.19 and 37.0 ± 1.53 nmol/g.tissue). On the other hand, mice treated with ketamine and NAC exhibited a 20.6% decrease in MDA levels in the cerebral cortex, compared to the ketamine only group (28.15 ± 0.74 vs. 35.44 ± 1.62 nmol/g.tissue) (Figure 1).

The administration of clozapine or haloperidol to ketamine-treated mice had no significant effect on brain MDA levels. NAC given to ketamine + clozapine-treated mice had no significant effect on brain MDA levels, compared to the ketamine + clozapine only group. NAC given to mice treated with ketamine + haloperidol caused a 23.5% decrease in MDA levels in the cortex but not in the rest of the brain tissue, compared to the ketamine + haloperidol only group (28.04 ± 1.53 vs. 36.67 ± 2.0 nmol/g.tissue) (Figure 1).

3.1.2. GSH Levels

NAC treatment alone had no significant effect on the brain GSH levels compared with saline-treated group. In mice treated with only ketamine, GSH levels were significantly decreased by 41% and 53.1% in the cerebral cortex and in the rest of the brain, respectively, as compared to the saline control values (3.78 ± 0.16 and 3.22 ± 0.11 vs. control values of 6.4 ± 0.23 and 6.87 ± 0.31 $\mu\text{mol/g.tissue}$). The administration of NAC to ketamine-treated mice was associated with 34.9% and 29% increases in GSH levels in the cerebral cortex and in the rest of the brain, respectively, as compared to the ketamine control group (6.26 ± 0.19 and 6.77 ± 0.12 vs. 3.78 ± 0.16 and 3.22 ± 0.11 $\mu\text{mol/g.tissue}$) (Figure 2).

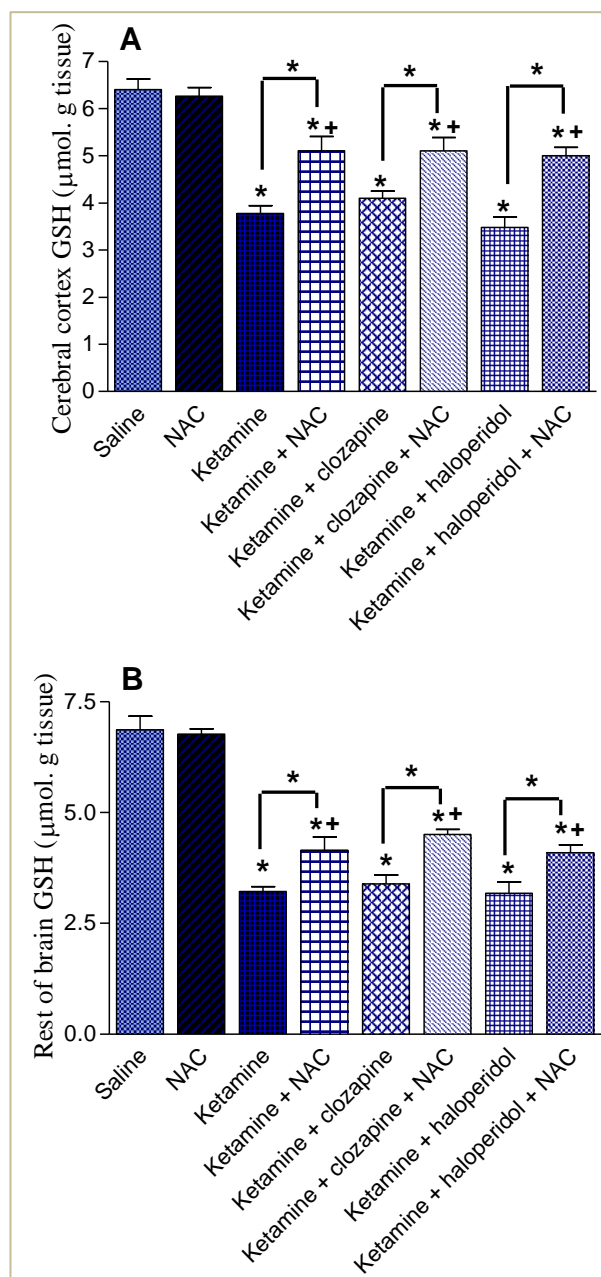
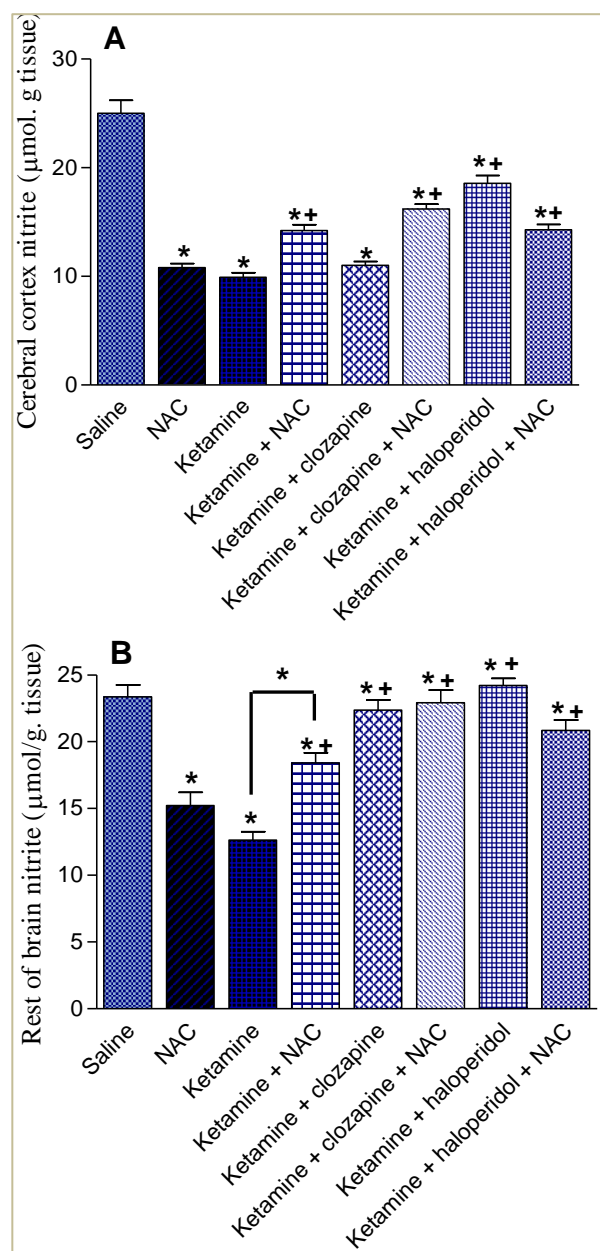


FIGURE 2. Effects of *N*-acetylcysteine (NAC) administration on the levels of reduced glutathione (GSH) in the cerebral cortex (A) and the rest of the brain tissue (B) in mice treated with ketamine alone or with antipsychotic drugs. Data are means \pm SEM, evaluation by one-way analysis of variance and Duncan test. *, $p < 0.05$ vs. saline-treated group and between other groups as indicated in the figure; +, $p < 0.05$ vs. ketamine only.



43.6% and 29% increments in GSH levels in both the cortex and the rest of the brain tissue, respectively, compared to the ketamine + haloperidol only group (5.0 ± 0.13 and 4.10 ± 0.17 vs. 3.48 ± 0.22 and 3.18 ± 0.26 μmol/g.tissue) (Figure 2).

3.1.3. Nitric Oxide Levels

NAC given to saline-treated mice resulted in 56.8% and 34.8% decrements in nitric oxide levels in both the cortex and the rest of the brain tissue, respectively, as compared to the saline control group (10.80 ± 0.39 vs. 25.0 ± 1.2 and 15.22 ± 1.0 vs. 23.36 ± 0.91 μmol/g.tissue). In mice treated with only ketamine, nitric oxide levels were significantly decreased by 60.3% and 45.9%, in both the cortex and the rest of the brain tissue, respectively, as compared to the saline control (9.92 ± 0.42 vs. 25.0 ± 1.2 and 12.64 ± 0.61 vs. 23.36 ± 0.91 μmol/g.tissue). The administration of NAC was associated with 34.9% and 29% increases in nitric oxide levels in the cerebral cortex and the rest of the brain, respectively, as compared to the ketamine control group (14.22 ± 0.53 vs. 9.92 ± 0.42 and 18.43 ± 0.65 vs. 12.64 ± 0.61 μmol/g.tissue) (Figure 3).

In mice treated with ketamine and clozapine, nitric oxide levels were significantly increased by 51.2%

FIGURE 3. Effects of *N*-acetylcysteine (NAC) administration on the levels of nitric oxide (nitrite) in the cerebral cortex (A) and the rest of brain tissue (B) in mice treated with ketamine alone or with antipsychotic drugs. Data are means \pm SEM, evaluated by one-way analysis of variance and Duncan test. *, $p < 0.05$ vs. saline-treated group and between other groups as indicated in the figure; +, $p < 0.05$ vs. ketamine only.

Clozapine or haloperidol given to ketamine-treated mice had no significant effect on brain GSH levels. NAC given to ketamine+ clozapine-treated mice resulted in 24.4% and 33% increases in GSH levels in the cortex and the rest of the brain tissue, respectively, compared to the ketamine + clozapine only group (5.10 ± 0.29 and 4.51 ± 0.11 vs. 4.10 ± 0.15 and 3.39 ± 0.20 μmol/g.tissue). The administration of NAC to ketamine + haloperidol-treated mice resulted in

and 84.9% in the cortex and the rest of the brain tissue, as compared to the ketamine control group (11.0 ± 0.36 vs. 9.92 ± 0.42 and 23.38 ± 0.75 vs. 12.64 ± 0.61 μmol/g.tissue). NAC given to ketamine and clozapine-treated mice had no significant effect on nitric oxide levels, as compared to the ketamine + clozapine only group. On the other hand, treatment with both ketamine and haloperidol exhibited 87.2% and 91.6% increments in nitric oxide levels, in both

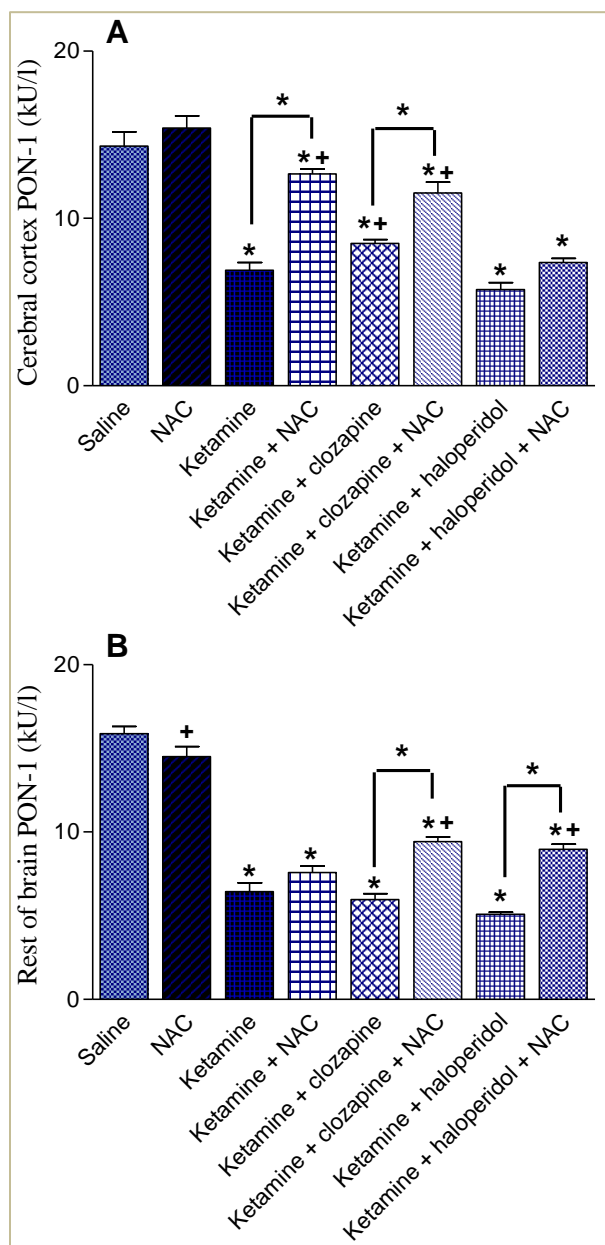


FIGURE 4. Effects of *N*-acetylcysteine (NAC) administration on paraoxonase-1(PON-1) activity in the cerebral cortex (A) and the rest of brain tissue (B) in mice treated with ketamine alone or with antipsychotic drugs. Data are means \pm SEM evaluated by one-way analysis of variance and Duncan test. *, $p < 0.05$ vs. saline-treated group and between other groups as indicated in the figure; +, $p < 0.05$ vs. ketamine only.

the cortex and the rest of the brain tissue, respectively, as compared with the ketamine only group (18.57 ± 0.73 vs. 9.92 ± 0.42 and 24.22 ± 0.55 vs. 12.64 ± 0.61 $\mu\text{mol/g.tissue}$). NAC given to ketamine and haloperidol-treated mice, the level of nitric oxide in the cortex decreased by 23% as compared to the ketamine + haloperidol only group (14.29 ± 0.5 vs. 18.57 ± 0.73 $\mu\text{mol/g.tissue}$) (Figure 3).

3.1.4. PON-1 Activity

NAC given to saline-treated mice had no significant effect on PON-1 activity in the cerebral cortex or in the rest of the brain tissue (15.41 ± 0.72 vs. 14.33 ± 0.85 and 14.50 ± 0.61 vs. 15.87 ± 0.44 kU/l). Ketamine, however, caused 51.8% and 59.4% decrements in PON1 activity in these brain regions, respectively, as compared to the saline control group (6.9 ± 0.48 vs. 14.33 ± 0.85 and 6.44 ± 0.53 vs. 15.87 ± 0.44 kU/l). Meanwhile, the administration of NAC to ketamine-treated mice resulted in 83.5% and 17.8% increases in PON-1 activity in the cerebral cortex and the rest of the brain tissue, respectively (12.66 ± 0.30 vs. 6.9 ± 0.48 and 7.59 ± 0.39 vs. 6.44 ± 0.53 kU/l) (Figure 4).

Mice given both ketamine and clozapine showed a significant increase in PON-1 activity in the cortex by 23.3% compared with the ketamine only group (8.51 ± 0.23 vs. 6.9 ± 0.48 kU/l). There were no significant changes in PON-1 activity in the rest of the brain tissue. NAC given to ketamine and clozapine-treated mice increased PON-1 activity by 35.4% and 57.7% in the cortex and the rest of the brain tissue, respectively, as compared with the ketamine and clozapine only group (11.52 ± 0.66 vs. 8.51 ± 0.23 and 9.43 ± 0.27 vs. 5.98 ± 0.35 kU/l) (Figure 4).

Mice treated with both ketamine and haloperidol exhibited 16.5% and 20.8% decrements in PON-1 activity in both the cortex and the rest of the brain tissue, respectively, as compared with the ketamine only group (5.76 ± 0.41 vs. 6.9 ± 0.48 and 5.10 ± 0.11 vs. 6.44 ± 0.53 kU/l). This decline in PON-1 activity was alleviated by treatment with NAC which increased PON-1 activity in these brain regions by 28.1% and 76%, respectively, compared with the ketamine and haloperidol only group (7.38 ± 0.23 vs. 5.76 ± 0.41 and 8.97 ± 0.31 vs. 5.10 ± 0.11 kU/l) (Figure 4).

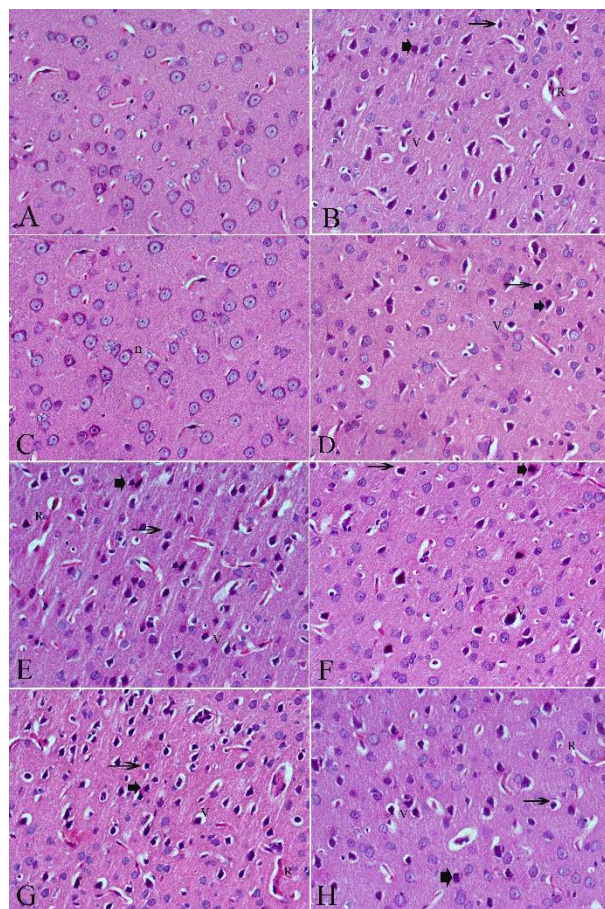


FIGURE 5. Representative photomicrographs of sections of the rat cortex. (A) Vehicle-control group; (B) Ketamine; (C) NAC; (D) Ketamine and NAC; (E) Ketamine and clozapine; (F) Ketamine, clozapine, and NAC; (G) Ketamine and haloperidol; (H) Ketamine, haloperidol, and NAC. Abbreviations: N, normal neurons; arrowhead, pyknotic nuclei; arrow, apoptotic nuclei; V, perineuronal vacuolations; R, red neurons.

3.2. Histopathological Results

3.2.1. Cerebral Cortex

In the vehicle-treated group, neurons in the cortex were arranged in neat rows with abundant cytoplasm and round basophilic nuclei (**Figure 5A**). Sections from ketamine-treated mice showed structural dam-

age, pink shrunken neurons, and apoptotic nuclei surrounded by perineuronal vacuolations. Moreover, a small number of red neurons were noted (**Figure 5B**). Microscopic investigation of the cortex of mice treated with NAC showed normal structure of neurons and the surrounding cells like control (**Figure 5C**). Mice treated with ketamine and NAC showed normal neurons (**Figure 5D**).

The cortex in mice treated with ketamine and clozapine showed neurodegeneration in the form of distorted neuronal morphology, and pyknotic and apoptotic nuclei surrounded by perineuronal vacuolations (**Figure 5E**). These changes were ameliorated by NAC with neurons being more or less like normal. Slight degeneration was found and loss of the number of neurons could be seen (**Figure 5F**). Sections from mice given both ketamine and haloperidol showed extensive neuronal damage (**Figure 5G**) which was markedly improved by the treatment with NAC. Neurons appeared more or less like normal although some pyknotic and apoptotic neurons were seen (**Figure 5H**).

3.2.2. Striatum

The striatum of the vehicle-treated group showed normal appearance (**Figure 6A**). Sections from ketamine-treated rats showed distortions in cellular architecture, and pyknotic and apoptotic nuclei surrounded by perineuronal vacuolations. Pink shrunken neurons were seen (**Figure 6B**). The striatum of mice given only NAC was similar to that of the vehicle-treated group (**Figure 6C**). Mice given ketamine and NAC showed improvement in the pathological changes compared to the ketamine only group. There were normally appearing neurons with round nuclei although mild pyknotic and apoptotic nuclei surrounded by perineuronal vacuolations and a number of red neurons could also be observed (**Figure 6D**).

Sections from mice treated with ketamine and clozapine revealed distorted neuronal morphology, pyknotic and apoptotic nuclei surrounded by perineuronal vacuolations, and red neurons (**Figure 6E**). The administration of NAC resulted in improved histological picture with normal neurons although mild pyknotic and apoptotic nuclei surrounded by perineuronal vacuolations and a number of red neurons are still seen (**Figure 6F**). The striatum of mice treated with ketamine and haloperidol showed loss of

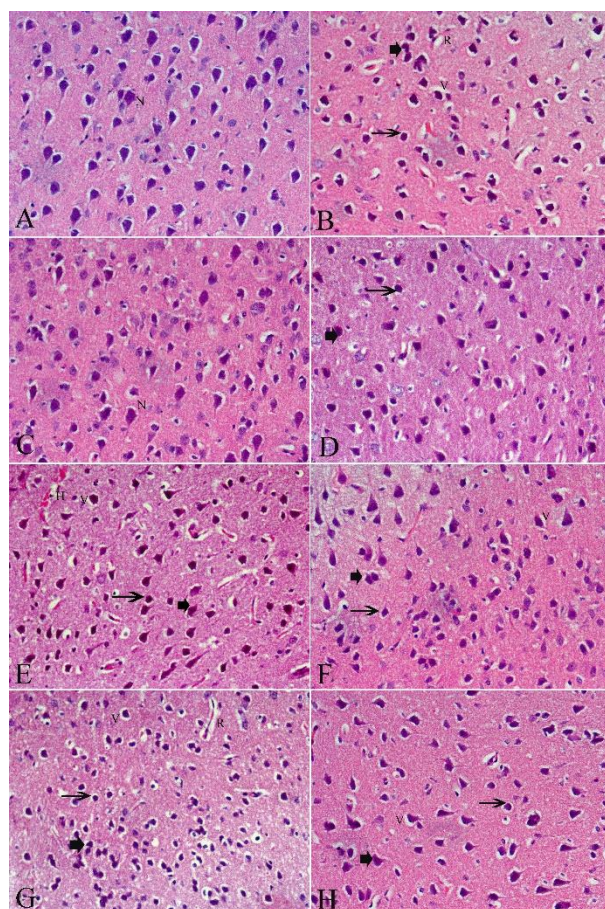


FIGURE 6. Representative photomicrographs of sections of the rat striatum. (A) Vehicle-control; (B) Ketamine; (C) NAC; (D) Ketamine and NAC; (E) Ketamine and clozapine; (F) Ketamine, clozapine, and NAC; (G) Ketamine and haloperidol; (H) Ketamine, haloperidol, and NAC. Abbreviations: N, normal neurons; arrowhead, pyknotic nuclei; arrow, apoptotic nuclei; V, perineuronal vacuolations; R, red neurons.

pigmented neurons. Cells were smaller and shrunken as compared to the control, indicating occurrence of apoptosis. In addition, pyknotic nuclei and perineuronal vacuolations were observed (**Figure 6G**). These pathological changes were ameliorated by treatment with NAC with neurons appearing more or less like normal although some pyknotic and apoptotic neurons were still seen (**Figure 6H**).

4. DISCUSSION

Ketamine, a dissociative anesthetic and an NMDA antagonist, has been shown to cause schizophrenia-like symptoms in humans and is widely used in rodents to create a condition mimicking schizophrenia [28, 30]. Oxidative stress has been strongly implicated in the development of schizophrenic symptoms and evidence of lipid peroxidation and protein oxidation/nitration has been found in the plasma of schizophrenic subjects [5, 9, 36, 37]. Here we studied oxidative stress in the brain of mice treated with ketamine alone or with the antipsychotic drugs clozapine and haloperidol. We also investigated the possible modulatory effect of the GSH precursor NAC. Our results show that ketamine increases brain oxidative stress which is in accordance with previously published data [38–41]. The repeated injection of the drug caused a significant increase in the brain lipid peroxidation end product MDA, indicating increased free radical generation and subsequent attack on membrane polyunsaturated fatty acids [42, 43]. The latter conclusion is supported by the observed decrease in brain concentration of GSH, a free radical scavenger and the most important antioxidant in the brain tissue. The tripeptide glutathione (γ -glutamylcysteinylglycine) is found in the cell mainly in its reduced form (GSH) and is subject to oxidation by free radicals and reactive oxygen/nitrogen metabolites to glutathione disulfide (GSSG) which is then reduced back to GSH by the enzyme glutathione reductase using NADPH. GSH is an effective scavenger of such reactive species as hydroxyl radicals, lipid peroxyl radicals, and peroxynitrite [44, 45]. It is likely therefore that the loss of GSH in the brain of ketamine-treated mice is the result of its consumption by reactive oxygen metabolites. Other studies also found increased oxidative stress indicated by increased lipid and protein oxidation markers as well as decreased antioxidants, such as GSH, superoxide dismutase activity, and catalase activity in the brain of rodents following single or repeated ketamine injection [38–41, 46].

The results of this study also indicate a marked decrease in brain nitric oxide content following ketamine injection which is in agreement with our previous observations [40, 41]. Ketamine given at a single dose of 30 mg/kg resulted in a significant decrease in the levels of nitric oxide in the cortex and striatum of mice treated with saline or with lipopoly-

saccharide endotoxin [40]. The repeated injection of the drug (30 mg/kg for 2 weeks) caused a significant depression of nitric oxide in the cortex and in the rest of the brain tissue [41]. Other researchers found decreased nitrite levels in the prefrontal cortex, hippocampus, and striatum of mice treated with ketamine for two weeks [46]. Ketamine inhibited the release of nitric oxide from alveolar macrophages and cultured microglia after stimulation with lipopolysaccharide endotoxin *in vitro* [47, 48]. This decrease in nitric oxide observed after ketamine treatment appears to be due to an inhibitory action on nitric oxide synthases [47, 49] and this action could be involved in the antidepressant effect of the drug [49, 50].

In this study and consistent with our earlier observations [40, 41], there was a significantly decreased brain PON-1 activity following ketamine administration. The PON1 enzyme is a calcium-dependent esterase and lactonase that is synthesized by the liver and has an important role in the detoxification of the active metabolites of some organophosphate insecticides [51]. Moreover, PON-1 exerts antioxidative [52] and anti-inflammatory actions [53] that are particularly important in neurodegenerative disorders where there are inappropriately high levels of oxidative stress and neuroinflammation [54, 55]. In this context, reduced PON-1 activity has been found in the plasma of patients with Alzheimer's disease [56], multiple sclerosis, mild cognitive impairment [57], autism [58], and also in schizophrenic subjects on antipsychotic drugs [59]. PON-1 is sensitive to oxidative stress and could be inhibited by oxidants [60] which could provide an explanation to the decline in the enzyme activity in the brain of ketamine-treated rodents. Recovery of the enzyme activity was observed in experimental models of neurotoxicity following alleviation of brain oxidative stress [61], suggesting that the enzyme could be considered as an indicator of the redox state in the cell.

Several studies showed evidence of increased oxidative stress in the brain of rodents treated with haloperidol or clozapine. These studies indicated increased lipid peroxidation and nitric oxide along with decreased antioxidants like GSH and superoxide dismutase activity [62–65]. Clozapine increased the production of reactive oxygen metabolites in neutrophils of treated patients [23] while either clozapine or haloperidol caused the increased formation of reactive oxygen metabolites in the rat whole blood [66]. In schizophrenic subjects on clozapine, lower GSH

was present in the plasma compared with controls [25]. Oxidization of proteins related to energy metabolism and mitochondrial function was also seen after clozapine treatment in lymphoblastoid cell lines [24]. In the present study, the administration of haloperidol or clozapine to ketamine-treated mice was not associated with an increase in lipid peroxidation or a decrease in the level of GSH or PON-1 activity beyond that of ketamine alone. Increased nitric oxide was, however, observed after ketamine and either haloperidol or clozapine. On the other hand, the administration of antipsychotics, especially haloperidol, led to worsening of the neurodegeneration caused by ketamine in both the cerebral cortex and striatum.

There is evidence to suggest a benefit from the GSH precursor NAC as an adjunct treatment in schizophrenia [16]. Here we showed that NAC given to ketamine-treated mice was able to alleviate the ketamine-induced biochemical alternations, namely, the increase in brain lipid peroxidation and the decline in GSH and PON1 activity. Interestingly, although NAC given to saline-treated mice resulted in a significant decrease in brain nitric oxide, NAC treatment led to a significant increase in brain nitric oxide levels in ketamine-treated mice. Moreover, NAC given to mice treated with ketamine and or haloperidol was shown to decrease lipid peroxidation in the cortex. It also increased the GSH level and PON1 activity in mice given ketamine and either clozapine or haloperidol. These observations could be interpreted as a result of a therapeutic effect for the drug and suggest that NAC by supplying cysteine and replenishing brain GSH antagonizes the biochemical changes induced by ketamine in the brain. The biochemical results are confirmed by the histopathological examination of the brain that revealed marked amelioration of the pathological changes caused by ketamine alone or in combination with the antipsychotic drugs. NAC provides the sulfur amino acid cysteine, the rate limiting amino acid for GSH synthesis [67]. It is also a scavenger of hydrogen peroxide and hypochlorous acid [68]. NAC was shown to decrease death of cerebellar granule neurons caused by the lipid peroxidation product 4-hydroxynonenal via restoring intracellular GSH and mitochondrial membrane potential [69]. In rats with traumatic brain injury, NAC was shown to restore brain GSH levels and mitochondrial electron transfer and energy coupling capacity. The findings of the

present study suggest the usefulness of NAC add-on therapy in preventing oxidative stress and further neuronal injury during the treatment with antipsychotic drugs.

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