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Neuroprotective effect of naphtha[1,2-*d*]thiazol-2-amine in an animal model of Parkinson's disease

FAIZUL AZAM[1], SANDEEP KUMAR BARODIA[2], TARIQUE ANWER[3], & M. M. ALAM[3]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Seventh of October University, PO Box 2873, Misurata, Libya,* [2]*Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India, and* [3]*Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India*

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Abstract

Increased oxidative stress has been implicated in the pathogenesis of dopaminergic neurodegeneration leading to the development of Parkinson's disease. In this study, we investigated whether naphtha[1,2-*d*]thiazol-2-amine (NTA) may ameliorate haloperidol-induced catalepsy and oxidative damage in mice brain. Haloperidol-induced catalepsy was measured with the standard bar test. The extent of oxidative stress has been evaluated by measuring levels of MDA, GSH and activities of antioxidant enzymes (SOD and GSH-Px) from brain homogenate. Haloperidol treatment significantly induced the catalepsy as observed from increased descent time measured in the bar test. Pretreatment with NTA significantly reduced the catalepsy induced by haloperidol in a dose-dependent manner. The elevated level of MDA in haloperidol-treated mice was significantly decreased by NTA pretreatment. The decreased level of GSH as well as SOD and GSH-Px activities in haloperidol-treated mice were significantly increased by NTA pretreatment. NTA reduces the oxidative stress allowing recovery of detoxifying enzyme activities and controlling free radical production, suggesting a potential role of the drug as an alternative/adjuvant drug in preventing and treating the neurodegenerative diseases, such as Parkinson's disease.

Keywords: *Parkinson's disease, Oxidative stress, Neuroprotection, Naphtha[1, 2-d]thiazol-2-amine, haloperidol*

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system, in which the cell damage mainly occurs in the area of the substantia nigra and striatum [1]. The underlying cause of this selective cell death is not understood, although several hypotheses have been advanced [2].

The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron [3]. The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. The vast majority of energy is used by the neurons [4]. Due to negligible glutathione (GSH)-producing capacity of neurons, the brain has a limited capacity to detoxify reactive oxygen species (ROS). Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, are most susceptible to oxidative stress. Antioxidant enzymes and low molecular weight antioxidants: the indirect-acting antioxidants (e.g. chelating agents) and direct-acting compounds (e.g. GSH, NADPH and exogenous agents from dietary sources: ascorbic acid, lipoic acid, polyphenols and carotenoids) are required for neuronal survival [5]. In addition, GSH depletion [6] as well as a decreased activity of several antioxidant enzymes, viz. superoxide dismutase (SOD) [7] and glutathione peroxidase (GSH-Px) [8] have been implicated in the pathogenesis of many neurological diseases such as PD. Also, elevated concentrations of free radicals and resultant oxidative

Correspondence: F. Azam, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Seventh of October University, PO Box 2873, Misurata, Libya. Tel: 218 91 3300265; 218 92 7677641. Fax: 218 51 628149. E-mail: azamfaizul@yahoo.co.in; faizulazam@gmail.com

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damage, such as lipid peroxidation (LPO) have been repeatedly demonstrated in PD and other neurodegenerative disorders [9].

The current treatment of PD is based on dopamine replacement therapy (levodopa), inhibition of monoamine oxidase (e.g. Selegiline), dopamine receptor agonists (e.g. bromocriptine and apomorphine) and antichloinergics (e.g. benztrophine, orphenadrine). Although the introduction of levodopa therapy in particular revolutionized the management of PD, the immediate or delayed side effects this treatment can cause, the most invalidating being dyskinesia, have prompted the search for alternative pharmacological strategies that reduce the incidence of this neurodegenerative disease by protecting or regenerating dopaminergic neurons [10]. The progressive neurodegeneration in PD is not halt/slow down by the currently used drug therapies. Hence, current researches are focusing on finding therapies which could help in preventing/delaying the ongoing neurodegeneration in PD [11].

The thiazoles have emerged as important target molecules due to their pharmacological properties such as antioxidant [12], anti-inflammatory [13], antimicrobial [14] and noteworthy, as drugs for treating neurodegenerative disorders [15]. For example, Pramipexole (Figure 1), an aminobenzothiazole analogue is a dopamine D_2/D_3 receptor agonist currently in clinical use for the treatment of PD [16]. It has been reported that pramipexole acts as a scavenger of ROS, based on the findings of an *in vivo* microdialysis study of the rat striatum [17]. The Protective effect of pramipexole against H_2O_2-induced PC12 cell death is also reported in the literature [18]. Lubeluzole (Figure 1), a novel benzothiazole compound which currently has tested antiischemic in clinical and preclinical studies has been shown to attenuate growth of ischemic damage as well as its density in the periphery of a photochemically induced neocortical infarct in rats [19]. It is a NOS inhibitor, which in addition decreases glutamate release and blocks sodium and calcium channels [20,21]. Another example of an analogue with an aminothiazole moiety is riluzole (Figure 1), a novel neuroprotective drug that is approved worldwide for the treatment of amyotrophic lateral sclerosis and is active in a wide variety of experimental models of neurodegenerative diseases, including toxin-induced models of PD in rodent and primate [22–25]. Furthermore, our recent study using the pentylenetetrazole model of oxidative stress demonstrates that administration of derivatives of naphtha[1,2-*d*]thiazol-2-amine (NTA, Figure 1) dramatically reduces MDA formation as well as normalizes the SOD and GSH-Px activity levels in mouse brain homogenate suggesting their antioxidant/neuroprotective properties [26].

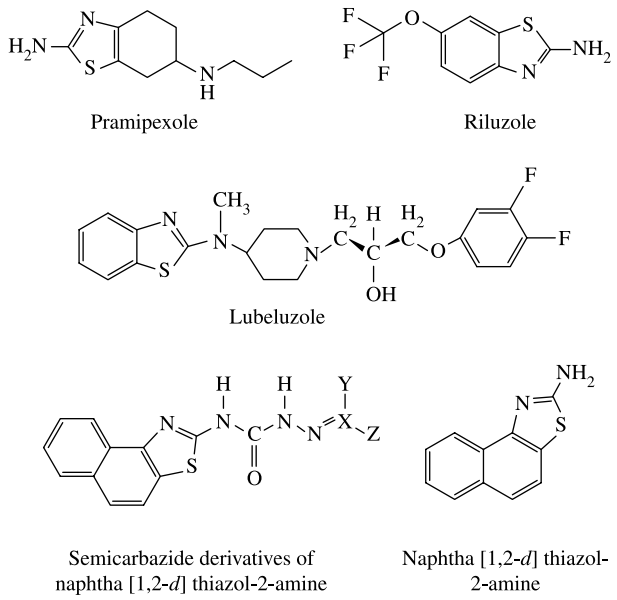

Figure 1. Structure of naphtha[1,2-*d*]thiazol-2-amine and some cited molecules.

In view of the above, the present study was designed to examine whether NTA ameliorated catalepsy induced by haloperidol and modified the effect of neuroleptic on LPO and GSH level as well as the antioxidant enzymes (SOD and GSH-Px) in mouse brain to predict their neuroprotective activity for the treatment of neurodegenerative disorders such as PD. We have presented some part of this work as an abstract [27].

Materials and methods

Chemistry

Synthetic starting material, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Aldrich Chemical Co. and Merck Chemical Co. The progress of the reactions was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets (Merck) using chloroform/methanol 95/5 as eluent; UV light and iodine vapours were used for detection. IR spectra were recorded, as KBr pellets, on a Schimadzu 8201 PC FT-IR spectrophotometer. The mass spectra were recorded on Jeol SX-102 (FAB). ^{1}N NMR spectra, in DMSO-d_6 and $CDCl_3$ solution, were recorded on a Bruker DRX-300 instrument at 298 K. Chemical shifts were reported as ppm relative to TMS as internal standard. Melting points (°C) were determined with an open glass capillary tube and are uncorrected. Elemental analyses were performed on Elementar Vario EL III instrument.

Synthesis of naphtha[1,2-d]thiazol-2-amine. It was synthesized according to the method of Azam et al. [26]. Briefly, to *N*-naphthylthiourea (0.05 mol) in 100 mL glacial acetic acid was added bromine (0.05 mol) in 10 mL glacial acetic acid at 10°C during 1 h. After being stirred at room temperature for 4 h, the reaction mixture was diluted with hot water, the solid

impurities were filtered off and the filtrate was basified with NH_4OH when a soft base crystallizeable from acetonitrile (5.8 g, 58%), m.p. 191°C, was obtained.

Animals and drugs

Adult male pathogen-free Swiss albino mice weighing 18-25 g, were used. All animal experimentation was conducted in accordance with the National Guidelines on the 'Proper Care and Use of Animals in Laboratory Research' (Indian National Science Academy, New Delhi, 2000) and was approved by the Animal Ethics Committee of the Institute. The procedures adhered to the NIH Guidelines for the Care and Use of Laboratory Animals. SCH 58261 (Sigma-Aldrich) was injected i.p. in dose of 5 mg/kg. Haloperidol (Sigma-Aldrich) was administered in dose of 5 mg/kg i.p. NTA was administered at 10, 30 and 100 mg/kg i.p. Caffeine (Sigma-Aldrich) was injected i.p. in dose of 10 mg/kg. All the drugs were suspended in 0.5% gum acacia in redistilled water and administered at a volume of 0.1 mL/100 g.

Measurement of catalepsy

Haloperidol-induced catalepsy was measured with the standard bar test [28], in a wooden chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal metal bar (diameter, 0.4 cm; length, 10.5 cm) fixed at 9 cm above the floor, and at 4 cm from the back of the box. All experiments were carried out between 8:00 and 15:30 h in a room with controlled temperature (23 ± 1°C), and light intensity of 20 lux. Mice were divided into seven groups with six animals in each group. Group 1: 0.5% gum acacia in redistilled water; group 2: haloperidol; groups 3, 4, 5, 6 and 7 received 10 mg/kg NTA, 30 mg/kg NTA, 100 mg/kg NTA, 5 mg/kg SCH 58261 and 10 mg/kg caffeine respectively 30 minutes prior to haloperidol injection. Animals were used only once. Catalepsy was measured every 15 min during the whole session that lasted 4 h after haloperidol injection. To assess whether the repeated handling of animals could have any influence on catalepsy intensity over time [28], the bar test was performed in groups of mice that were injected only with the vehicle in which haloperidol was dissolved (group 1).

To measure catalepsy, the mouse was gently lifted until its forepaws firmly grasped the metal bar. Then, the mouse body was released and simultaneously a stop-watch was started. The time elapsed until the animal released both forepaws from the bar, up to a maximum of 300 s, was defined as the descent time. The sum of the descent time values measured every 15 min during the 4 h after haloperidol or vehicle was defined as the cumulative descent time (CDT[4h]). The mean CDTs measured in animals treated by the vehicle in which haloperidol was dissolved were subtracted from the mean CDTs recorded in mice treated with haloperidol. This difference was taken as 100% of catalepsy, and served as a reference value to calculate the percent inhibition of drugs on catalepsy intensity.

Biochemical evaluation

Mice were sacrificed by decapitation 4 h after the last injection. The brains were quickly removed and were washed twice with ice-cold saline solution, placed into glass bottles, labeled, and stored in a deep freeze (−30°C) until processing (maximum 10 h). Tissues were homogenized in four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T18 Basic, USA) after cutting up the brain into small pieces with scissors (for 2 min at 5000 rpm). Malondialdehyde (MDA) and protein levels were carried out at this stage. The homogenate was then centrifuged at 10500 × g for 20 min to remove nuclear debris. Clear supernatant fluid was taken and GSH-Px activity was carried out in this stage. The supernatant solution was then extracted with an equal volume of an ethanol/chloroform mixture (5:3, v/v). After centrifugation at 5000 × g for 30 min, the clear upper layer (the ethanol phase) was taken and used in the SOD activity. All preparation procedures were performed at +4°C.

LPO assay. The extent of LPO in brain homogenate was determined by measuring the release of thiobarbituric acid reactive substance in terms of MDA equivalent using a molar extinction coefficient of 1.56×10^5/min/cm as described by Ohkawa et al. [29]. Briefly, the homogenate was centrifuged at 3000 × g for 15 min and supernatant was used for assay. Samples of 0.1 mL homogenate was mixed with 0.2 mL of 8.1% SDS, 1.5 mL 20% glacial acetic acid and 1.5 mL of 0.8% thiobarbituric acid. Following these additions, tubes were mixed and heated at 95°C for 1 h on a water bath and cooled under tap water before mixing 1 mL of distilled water and 5 mL mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200 × g for 10 min. The amount of MDA formed was measured by the absorbance of upper organic layer at a wavelength of 532 nm in Perkin Elmer spectrophotometer using appropriate controls. The results are expressed as nmol MDA/mg protein.

GSH determination. The amount of GSH in brain was measured according to the method of Sedlak and Lindsay [30]. Briefly, brain tissue was deproteinized with an equal volume of 10% TCA and was allowed to stand at 4°C for 2 h. The contents were centrifuged at 2000 × g for 15 min. The supernatant was added to 2 mL of 0.4 M Tris buffer (pH 8.9) containing 0.02 M EDTA (pH 8.9) followed by the addition of 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid). Finally,

the mixture was diluted with 0.5 mL of distilled water, to make the total mixture to 3 mL and absorbance was read in a spectrophotometer at 412 nm and results are expressed as μg GSH/gm Tissue.

SOD activity determination. Cu,Zn-SOD activity was determined according to the method of Sun et al. [31]. In this method, a xanthine–xanthine oxidase complex produces superoxide radicals, which react with nitrobluetetrazolium (NBT) to form the formazan compound. In brief, a reactive was prepared with 0.1 mM of xanthine, 0.1 mM of EDTA, 50 mg of bovine serum albumin, 25 μM of NBT and 40 mM of Na_2CO_3 (pH 10.2). To 2.45 mL of reactive was added 0.5 mL of a ethanol/chloroform (5:3, v/v) extract, previously prepared from brain homogenate. Subsequently, 50 μl of 9.9 nM of xanthine oxidase solution was added, the mixture was kept in a water bath of 25°C for 20 min, and the reaction was terminated using 1 mL of $CuCl_2$. The absorbance of the samples was read at 560 nm. In the control sample the amount of the ethanol supernatant was replaced by equivalent volume of PBS buffer. One unit SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction to formazan. SOD activity was expressed as U/mg protein.

GSH-Px activity determination. The GSH-Px activity was measured by the method of Paglia and Valentine [32]. The enzymatic reaction was conducted in 3 mL quartz cuvettes of 1 cm path length in a Perkin-Elmer spectrophotometer. Each 3 mL assay volume contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 U glutathione reductase and 1 mM reduced glutathione. The sample (0.2 mL of the tissue homogenate), after its addition, was allowed to equilibrate for 5 min at 25°C. The reaction was initiated by adding 0.1 mL of 2.5 mM H_2O_2. Changes in absorbance were recorded at 340 nm for 5 min. Values were expressed as units of NADPH oxidized to NADP by using the extinction coefficient of 6.22 mM^{-1} cm^{-1} at 340 nm. All samples were assayed in duplicate. GSH-Px activity was expressed as units per gram protein.

Total protein determinations. Total protein concentration of brain homogenates was determined by folin–phenol reaction as described by Lowry et al. [33]. The bovine serum albumin was used as a standard.

Statistical analysis

Data were expressed as the mean ± standard error (S.E) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The post hoc Bonferroni multiple comparison test was applied to identify significance among groups. $p < 0.05$ was considered to be statistically significant.

Results and discussion

Haloperidol-induced catalepsy

There are currently a number of pharmacological models that can reproduce many of the symptoms displayed in Parkinsonian patients such as bradykinesia, tremor, and rigidity. Catalepsy is a behavioral condition (characterized by the rigid state of a part or all of the muscle) that shares some similarity to human PD [34,35]. Fairly sustained catalepsy can be induced by the dopamine D_2 receptor block of haloperidol [35].

Haloperidol (5 mg/kg) produced a profound increase in catalepsy as shown by a progressive increase in the latency to step down the rod over time as compared with controls ($p < 0.001$) (Figure 2). Catalepsy induced by haloperidol was significantly and dose dependently

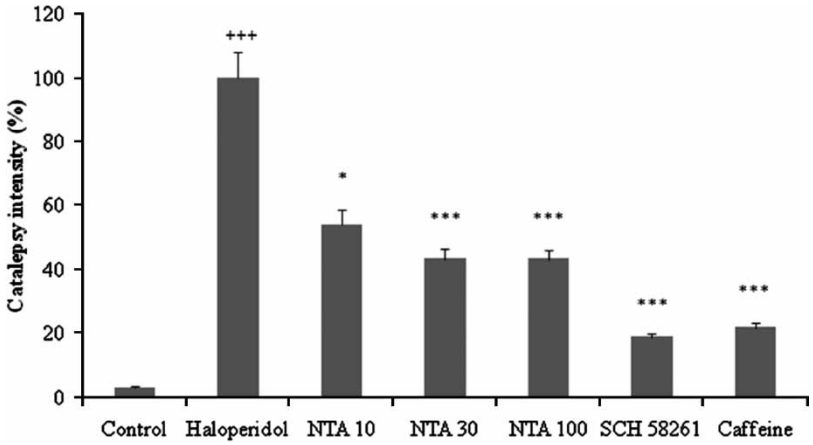

Figure 2. Effect of NTA and standard drugs on haloperidol-induced catalepsy in mice. Data is presented as % catalepsy. The difference between mean CDT[4h] of haloperidol group and control group was taken as 100% catalepsy. Similarly, % catalepsy of NTA, SCH 58261 and caffeine treated groups were calculated. $^{+++}p < 0.001$ compared with the corresponding value for control mice. *$p < 0.01$ compared with corresponding value for haloperidol-treated mice. ***$p < 0.001$ compared with corresponding value for haloperidol-treated mice.

antagonized by the injection of NTA (10, 30 and 100 mg/kg) throughout the period of observations, starting from 15 min till 240 min. The reduction in cataleptic scores with caffeine (10 mg/kg, i.p.; $p < 0.001$) and SCH 58261 (5 mg/kg, i.p.; $p < 0.001$) was also significant ($p < 0.001$) throughout the period of observations (Figure 2). The descent time observed at different time intervals is presented in Table I. Considering the catalepsy produced by haloperidol after 4 h as 100%, the catalepsy observed with NTA 10, 30 and 100 mg/kg were 54.28%, 43.4% and 43.32% respectively. SCH 58261 produced 18.91% catalepsy while it was 21.78% in case of caffeine.

The results obtained with standard drugs are consistent with previous studies demonstrating that systemic administration of these drugs can reverse the catalepsy that were induced by haloperidol [36]. In addition, these results involving catalepsy are consistent with those of studies that employed other measures of motor dysfunction, including haloperidol-induced rigidity [37], deficits in locomotion and drug-induced tremulous jaw movements, which are used as an animal model of parkinsonian tremor [38].

Haloperidol-induced oxidative stress

Although the mechanism of dopaminergic neuronal death in PD has not been resolved, it is widely accepted that oxidative stress underlies the selective vulnerability of these neurons [39,40]. Chronic treatment of haloperidol is known to induce oxidative stress due to increased turnover of dopamine, and is thought to be responsible for its extrapyramidal side effects [41,42]. The oxidative stress and extrapyramidal side effects attenuate on increasing doses of haloperidol [43]. Haloperidol is cytotoxic to primary hippocampal neurons, C6 glioma cells and NCB20 cells [44]. It has been demonstrated that amyloid beta resistant cells were resistant to haloperidol toxicity, implying the role of free radicals in haloperidol-induced cell death [45]. Also, Bcl-2 prevents cell death caused by haloperidol, which also implicates free radicals as a cause of the cell death [46]. Furthermore, many preclinical and clinical studies have also proposed the production of ROS as causes of haloperidol-induced toxicity [47]. In addition, catabolism of dopamine by monoamine oxidase-B can produce large amounts of ROS, which can enter into cycles of free radical-generating reactions of the Fenton type with ferric ions present in large quantities in nigral cells [48].

LPO is a chain reaction between polyunsaturated fatty acids and ROS, and it produces lipid peroxides and hydrocarbon polymers that are highly toxic to the cell [49]. MDA is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore, used as a marker of LPO [50]. Haloperidol treatment in the present study induced LPO as indicated by a significant increase in brain MDA levels when compared with vehicle treated mice (Figure 3). Administration of NTA at the doses of 10, 30 and 100 mg/kg 30 minutes prior to haloperidol injection significantly reduced the extent of LPO when compared with haloperidol-alone treated mice. Adenosine A_{2A} receptor antagonists (Caffeine and SCH 58261) significantly reduced the effect of haloperidol on LPO. These results are supported by the evidence of elevated LPO in haloperidol-treated rats [41]. Furthermore, the increased LPO seen in the substantia nigra of PD patients bears witness to this high level of oxidative stress [51]. Such free radical mechanisms may thus play a key role in the pathological process in PD.

Under normal physiological conditions, it is estimated that up to 1% of the mitochondrial electron flow leads to the formation of superoxide ($O_2^{\cdot -}$), the primary oxygen free radical produced by mitochondria; and interference with electron transport can dramatically increase $O_2^{\cdot -}$ production. While these partially reduced oxygen species can attack iron and sulfur centres in a variety of enzymes, $O_2^{\cdot -}$ is rapidly converted within the cell to hydrogen peroxide (H_2O_2) by the SOD [52]. However, H_2O_2 can react with reduced transition metals, via the Fenton reaction, to produce the highly reactive hydroxyl radical ($^{\cdot}OH$) [53], a far more damaging molecule to the cell. The antioxidant enzymes catalase and GSH-Px detoxify H_2O_2 by converting it to O_2 and H_2O [54,55]. In addition, to help detoxify ROS, biological antioxidants, including GSH, α-tocopherol, carotenoids, and ascorbic acid, will react with most oxidants. Similarly, our results showed that a consistent decrease of GSH level (Figure 4), SOD (Figure 5) and GSH-Px (Figure 6) activities in haloperidol-treated mice is an indication of an impaired synthesis of GSH and degradation of antioxidant enzymes by free radicals during detoxification processes. It appears that increased levels of ROS are not detoxified in haloperidol-treated mice due to decreased efficiency of antioxidant enzymatic and non-enzymatic mechanisms, and may act as mediators of neuronal damage. NTA administered prior to haloperidol suppressed these effects, restoring GSH level and antioxidant enzymes (SOD and GSH-Px) activities to values almost similar to the controls.

Our results are consistent with those reported in the literature where the effect of the neuroleptics, clozapine, haloperidol, olanzapine and risperidone on the activities and contents of LPO and GSH as well as antioxidant enzymes in rat brains has been examined [56–58]. Numerous reports indicate that an excessive production of free radicals is associated with neuroleptic administration in rodents [59]. This abnormal free radical production is often related with a reduction of GSH content and detoxifying enzymes activity as well as higher thiobarbituric acid reactive substances as a result of increased LPO [60,61]. Such effects have also been observed in humans [62,63].

Table I. Mean descent time observed at different time intervals.

	Mean Descent Time (s) ± S.E.M.						
Time	Control	HAL	NTA 10	NTA 30	NTA 100	Caffeine	SCH 58261
15	5.2 ± 0.47	50 ± 4.2⋆	31.3 ± 3.91†	16.2 ± 1.62‡	16.3 ± 1.99‡	6.2 ± 0.60‡	5.8 ± 0.47‡
30	5.8 ± 0.47	85.67 ± 2.62⋆	40.5 ± 4.20†	24.8 ± 2.93‡	26.2 ± 3.07‡	7.7 ± 0.42‡	7 ± 0.51‡
45	5.7 ± 0.61	96.33 ± 2.84⋆	48.5 ± 5.59†	37.3 ± 3.35‡	36.5 ± 2.84‡	8.8 ± 0.47‡	8 ± 0.51‡
60	5.5 ± 0.67	108 ± 1.36⋆	64.7 ± 3.30†	48 ± 3.67‡	37.3 ± 3.12‡	13.3 ± 1.14‡	12.2 ± 0.87‡
75	5.3 ± 0.49	135.7 ± 4.24⋆	75.3 ± 3.35†	60.2 ± 3.04‡	58.7 ± 4.14‡	29.7 ± 2.52‡	21.2 ± 2.00‡
90	6.3 ± 0.42	157.2 ± 2.72⋆	84.7 ± 2.44†	65.8 ± 3.42‡	65.7 ± 2.98‡	35.2 ± 1.44‡	27.5 ± 0.56‡
105	5.5 ± 0.42	167.5 ± 2.56⋆	87.7 ± 2.47†	70.5 ± 2.17‡	72 ± 2.03‡	37.5 ± 1.76‡	30.5 ± 2.04‡
120	5.7 ± 0.66	175.3 ± 4.89⋆	92.8 ± 2.89†	75.2 ± 2.41‡	76.5 ± 1.74‡	40.8 ± 2.25‡	35.7 ± 2.80‡
135	6.3 ± 0.42	188.8 ± 8.87⋆	98.3 ± 4.02†	81 ± 1.94‡	81.3 ± 1.89‡	46.3 ± 2.75‡	40.7 ± 2.92‡
150	6.2 ± 0.6	221.2 ± 4.51⋆	107.5 ± 5.23†	88.7 ± 1.89‡	89.8 ± 2.77‡	49.7 ± 3.5‡	44 ± 3.84‡
165	6 ± 0.57	247.7 ± 4.66⋆	117.7 ± 4.85†	98.3 ± 2.77‡	97.3 ± 2.15‡	57.7 ± 3.33‡	51 ± 4.53‡
180	5.2 ± 0.4	269.7 ± 4.71⋆	133.8 ± 4.99†	105.7 ± 3.69‡	106.2 ± 3.60‡	64.2 ± 2.98‡	54.3 ± 3.87‡
195	5.2 ± 0.3	276.8 ± 7.16⋆	153.8 ± 4.42†	122.8 ± 4.02‡	123.2 ± 5.11‡	72.5 ± 2.88‡	64.5 ± 2.48‡
210	6.2 ± 0.47	280.2 ± 6.6⋆	170.2 ± 4.31†	137.5 ± 4.86‡	140 ± 2.85‡	84.2 ± 4.35‡	74.5 ± 3.30‡
225	5.8 ± 0.47	289.3 ± 5.47⋆	181.5 ± 3.14†	161.2 ± 3.25‡	158.2 ± 4.13‡	88.3 ± 3.61‡	83.7 ± 3.33‡
240	5.8 ± 0.54	292.7 ± 4.42⋆	204.8 ± 8.22†	178.8 ± 4.80‡	174.5 ± 5.36‡	92.3 ± 4.60‡	89.2 ± 2.05‡
Mean CDT[4h]	91.7 ± 0.10	3042 ± 20.15⋆	1601.5 ± 12.85†	1280.3 ± 11.82‡	1278 ± 11.52‡	642.34 ± 7.23‡	558 ± 6.81‡

Data is presented as mean descent time (s) ± S.E.M. Number of animals per group (n) = 6, HAL: Haloperidol (5 mg/kg), NTA 10: naphtha[1,2-*d*]thiazol-2-amine 10 mg/kg 30 min prior to HAL injection, NTA 30: naphtha[1,2-*d*]thiazol-2-amine 30 mg/kg 30 min prior to HAL injection, NTA 100: naphtha[1,2-*d*]thiazol-2-amine 100 mg/kg 30 min prior to HAL injection, Caffeine 10 mg/kg 30 min prior to HAL injection, SCH 58261 5 mg/kg 30 min prior to HAL injection. Data was analyzed by one-way ANOVA followed by Bonferroni post hoc analysis; ⋆$p < 0.001$ as compared with control group; †$p < 0.01$ as compared with haloperidol group; ‡$p < 0.001$ as compared with haloperidol group.

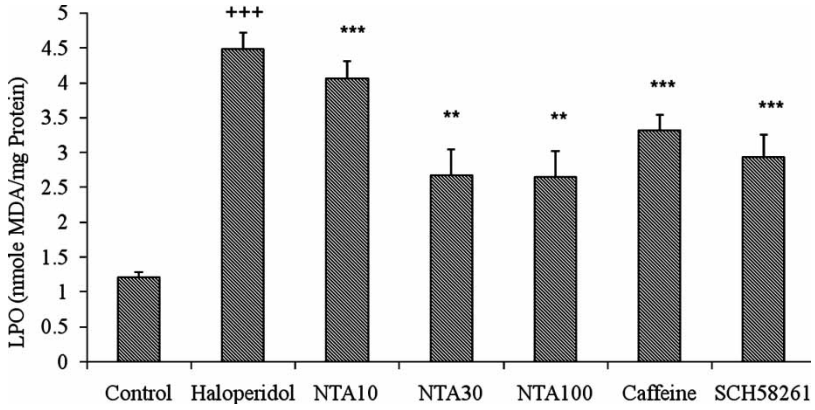

Figure 3. Effect of NTA and standard drugs on MDA activity in brain of mice treated with haloperidol. The data are expressed as mean ± S.E.M (n = 6). $^{+++}p < 0.001$ compared with the corresponding value for control mice. $^{**}p < 0.01$ compared with corresponding value for haloperidol-treated mice. $^{***}p < 0.001$ compared with corresponding value for haloperidol-treated mice.

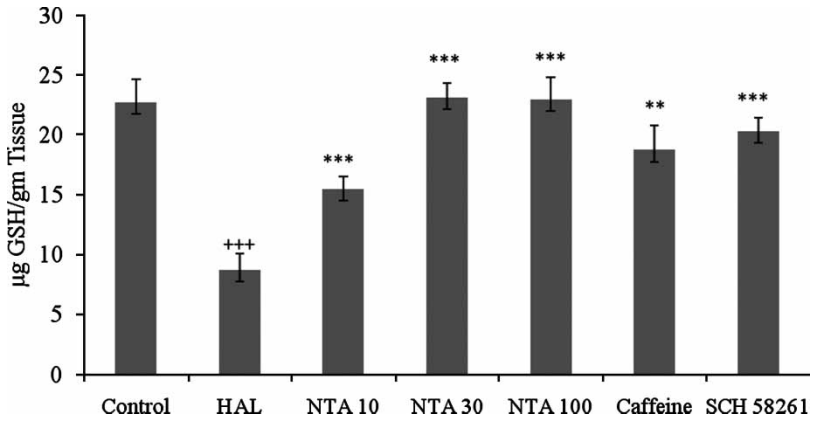

Figure 4. Effect of NTA and standard drugs on GSH content in mice brain treated with haloperidol. The data are expressed as mean ± S.E. (n = 6). $^{+++}p < 0.001$ compared with the corresponding value for control mice. $^{***}p < 0.001$ compared with corresponding value for haloperidol-treated mice. $^{**}p < 0.01$ compared with corresponding value for haloperidol-treated mice.

Antioxidants are a key to prevention and control of PD; many have reported that Parkinsonism was partially protected by the application of antioxidants [64–66]. Our results demonstrate that NTA has antioxidant effect on catalepsy model of PD, offering protection by enhancing GSH content and anti-

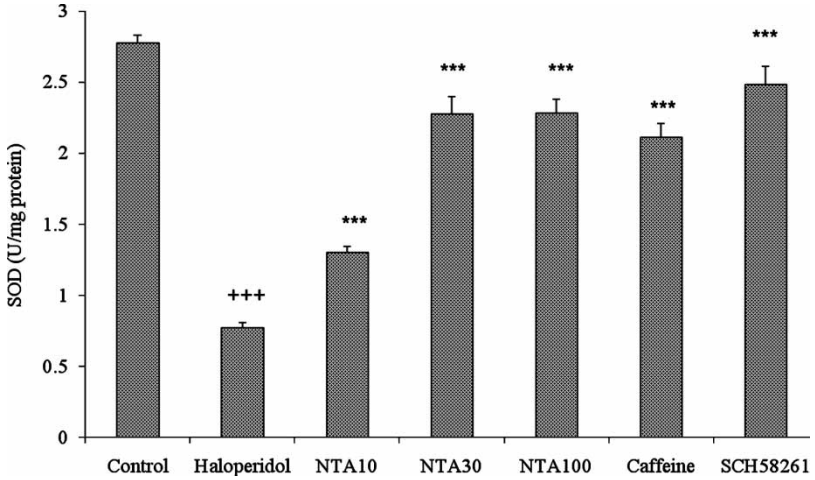

Figure 5. Effect of NTA and standard drugs on SOD activity in brain of mice treated with haloperidol. The data are expressed as mean ± S.E. (n = 6). $^{+++}p < 0.001$ compared with the corresponding value for control mice. $^{***}p < 0.001$ compared with corresponding value for haloperidol-treated mice.

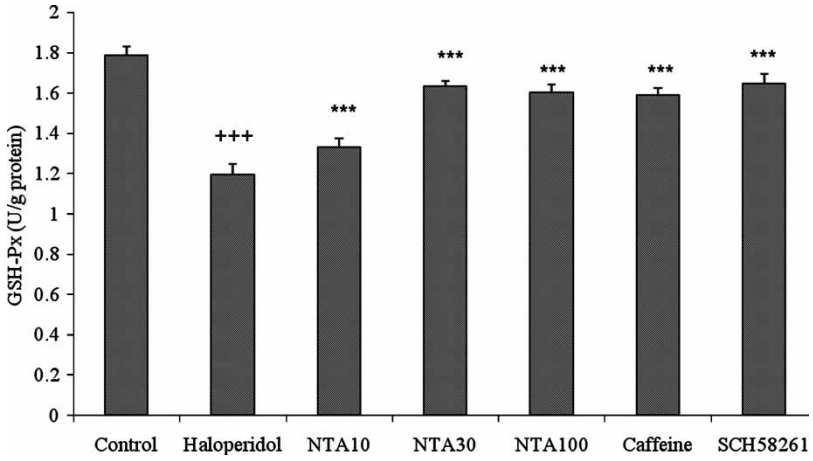

Figure 6. Effect of NTA and standard drugs on GSH-Px activity in brain of mice treated with haloperidol. The data are expressed as mean ± S.E. (n = 6). $^{+++}p < 0.001$ compared with the corresponding value for control mice. ***$p < 0.001$ compared with corresponding value for haloperidol-treated mice.

oxidant enzymes (SOD and GSH-Px) activities as well as decreasing LPO in mice treated with haloperidol. These findings extend the protection profile of NTA derivatives with beneficial effect proven in other model of oxidative stress reported by our research group [26].

Conclusion

We postulate that the observed effects of NTA are probably due either to its antioxidant or free radical scavenging properties leading to marked reduction of LPO and increased GSH content and antioxidant enzymatic activity in mice brain homogenate. However, the precise mechanisms of these antioxidant effects of NTA remain to be elucidated. Whatever the mechanisms, NTA may be potentially useful as a neuroprotective agent in the management of PD and other neurodegenerative disorders associated with oxidative stress. These novel pharmacological actions of NTA, in addition to its antioxidant activity, may be responsible for its clinical efficacy and deserve further testing in experimental research and clinical trials.

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