Innovations in Pharmaceuticals and Pharmacotherapy

IPP

www.innpharmacotherapy.com

eISSN: 2321–323X

Original Article

Pharmacological inhibition of PDE1 by vinpocetine attenuates 3-nitropropionic acid-induced behavioral and biochemical abnormalities in rats

Rahul Deshmukh*, P L Sharma

Neuropharmacology Division, I. S. F. College of Pharmacy, Moga-142001, Punjab, India.

Abstract

Enhancing cyclic nucleotide signaling by phosphodieterase inhibition (PDEs) has been reported to be beneficial in neurodegenerative disorders. The present study was designed to investigate the effect of vinpocetine (PDE1 inhibitor) in 3-nitropropionic acid (3-NP) induced experimental Huntington's disease. 3-nitropropionic acid was administered for 14 days (10 mg/kg i.p) in rats and these animals were treated with vehicle or different doses of vinpocetine (5, 10 and 20 mg/kg i.p). Changes in body weight, cognitive and motor behavior were assessed at different time points. Biochemically markers of oxidative stress, such as striatal glutathione and malondialdehyde levels were assessed terminally. Chronic administration of 3-NP produced significant decrease in body weight, showed marked abnormalities in cognitive and motor function and increased striatal oxidative stress. Vinpocetine dose dependently attenuated 3-NP induced behavioral and biochemical toxicity. Among the doses selected, vinpocetine at a dose of 10 mg/kg i.p was observed to be most effective in improving learning and memory in morris water maze and other motor functions such as grip strength, limb withdrawal and locomotor activity in rats. Further vinpocetine significantly attenuated oxidative stress in 3-NP treated rats. The above results suggesting that inhibition of PDE1 would be therapeutically beneficial in motor disorders including Huntington's disease.

Key words: Phosphodiesterase 1; Vinpocetine; Motor disorders; Huntington's disease; Oxidative stress

***Corresponding Author:** *Dr. Rahul Deshmukh, Associate Professor, Neuropharmacology Division ISF College of Pharmacy, Moga- 142001 Punjab, India. E-mail–login2rd@gmail.com

1. Introduction

Huntington's disease (HD) is a dominantly inherited human neurodegenerative disorder characterized by progressive motor impairment, involuntary movements, cognitive decline, and psychiatric disturbances leading to premature death [1]. HD is caused by a mutation encoding an abnormal expansion of CAG-encoded polyglutamine repeats in a protein called huntingtin (mhtt) [2]. Mutated htt has been reported to impair cyclic nucleotide (cAMP and cGMP) signaling and its downstream cAMP response element-binding protein (CREB)

transcriptional pathways, that has been hypothesized to play a critical role in HD pathology [3-12]. Moreover, decreased level cyclic nucleotides were observed in cerebrospinal fluid of HD patients [13]. These findings suggest that counteracting the decreased cyclic nucleotide signaling and loss of CREB-regulated transcription may be beneficial in treating HD.

Inhibition of phosphodiesterase (PDE) enzymes is a way to enhance second messenger mediated signaling and consequently influence the pathways involved in cognitive and motor functions. Phosphodiesterases are enzymes that break down cyclic nucleotides i.e. cAMP or cGMP or both. Type I phosphodiesterases (PDE1) are a family of Ca2+- calmodulin-modulated phosphodiesterases involved in the regulation of both cAMP and cGMP through their degradation (Bender and Beavo, 2006). PDE1 has been reported to be expressed in dopaminergic regions such as the striatum and nucleus accumbens [14,15] and the levels of PDE1B mRNA were found to be decreased in HD transgenic mice and in symptomatic HD patients, all provide evidence for an mhtt-dependent decrease in PDE1B expression [16-20]. Decreased PDE1 expression may occur either because of a direct effect of mhtt expression or represent a compensatory mechanism during disease progression but it is not yet clear.

Vinpocetine (14-ethoxycarbonyl-(3a,16a-ethyl)-14,15-eburnamine; Cavinton) is a synthetic derivative of the lesser periwinkle plant alkaloid vincamine and is widely used as a neuroprotective agent for the prevention and treatment of central nervous system disorders of cerebrovascular origin [21]. Vinpocetine (ethyl apovincaminate) a classic inhibitor of PDE1 activity [22] and has been is reported to induce significant rise in cyclic nucleotide levels and increase CREB phosphorylation [23-26] in rat brain. Vinpocetine has been reported to be beneficial in various neurodegenerative disorders, including stroke, Alzheimer's and Parkinson's disease (21,27,28) but it's potential in HD is yet to be identified. 3-Nitropropionic acid (3-NP) act by inhibiting mitochondrial complex II and closely mimics some of the main behavioral and pathologic features of HD pathology [29,30].

Moreover, 3-NP has also been demonstrated to cause decrease in striatal cyclic nucleotide levels and its downstream CREB mediated transcriptional activity [31,32]. In the present study we have investigated the effect of vinpocetine - a PDE1 inhibitor in 3-nitropropionic acid induced experimental Huntington's disease in rats.

2. Material and methods

2.1 Experimental Animals

The experiments were carried out in (5-6 months old) male Wistar rats (220-250 g) obtained from Central Animal House of I.S.F. College of Pharmacy, Moga, Punjab (India). They were kept in polyacrylic cages and maintained under standard housing conditions with 12h light/dark reverse cycle. The food in the form of dry pallets and water were made available *ad libitum.* All behavioural experiments were carried out between 10 AM and 4 PM. The protocol was reviewed and approved by the Institutional Animal Ethics Committee and the animal experiments were carried out in accordance with the Indian National Science Academy Guidelines for use and care of animals.

2.2 Drugs and Chemicals

3-Nitropropionic acid (3-NP), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was purchased from Sigma–Aldrich, USA. Vinpocetine was received as *ex-gratia* sample from covex pharma, Spain. 3-NP and vinpocetine were dissolved in 1% ascorbic acid in saline. All other chemicals used in the study were of analytical grade. Solutions of the drugs and chemicals were freshly prepared before use.

2.3 Experimental Procedure

3-NP was administered at a dose of 10mg/kg i.p for 14 days. 3-NP injected rats were treated with vehicle, vinpocetine (5, 10 and 20 mg/kg i.p) for 14 days 1h prior to 3-NP administration. The

volume of injection (0.5 ml/100 g) was kept constant throughout experimental groups. Animals were randomly divided into six groups (n=6) and all the observations were taken at one fixed time between 10:00 AM and 4:00 PM throughout the study.

2.5. Measurement of body weight

Animals' body weights were recorded on the first and last day of the experimentation, for calculation of percent change in body weight.

2.6. Behavioral assessment

2.6.1. Morris water maze test

Spatial learning and memory of animals were tested in a Morris water maze. It consisted of a circular water tank (180cm diameter, 60cm height) filled with water (25±1°C) to a depth of 40cm. A non-toxic water dispersible emulsion was used to render the water opaque. Four equally spaced locations around the edge of the pool (North, South, East, and West) were used as start points, which divided the pool into 4 quadrants. An escape platform (10cm in diameter) was placed in the pool 2cm below the surface of water. The escape platform was placed in the middle of one of the randomly selected quadrants of the pool and kept in the same position throughout the entire experiment (north-east for this study). Before the training started, the rats were allowed to swim freely into the pool for 60s without platform.

Animals received a training session consisting of 4 trials per session (once from each starting point) for 4 days (day 7, 8, 9 and 10), each trial having a ceiling time of 80s and a trial interval of approximately 30s. After climbing onto the hidden platform, the animals remained there for 30s before commencement of the next trial. If the rat failed to locate the hidden platform within the maximum time of 80s, it was gently placed on the platform and allowed to remain there for the same interval of time. The time taken to locate the hidden platform (latency in seconds) was measured. Twenty-four hours after the acquisition phase, a probe test (day 12) was conducted by removing the platform. Rats were allowed to swim freely in the pool for 60s and the time spent in target quadrant, which had previously contained the hidden platform, was recorded. The time spent in the target quadrant indicated the degree of memory consolidation which had taken place after learning [27].

2.6.2. Spontaneous locomotor activity

Each animal was tested for spontaneous locomotor activity on day 1, 8 and 15. Each animal was observed over a period of 5 min in a square closed arena equipped with infrared light sensitive photocells using a digital Actophotometer (INCO, India) [27].

2.6.3. Limb Withdrawl test

In this behavioral test, the animal was placed on a 20 cm high 30 cm × 30 cm Perspex platform containing four holes, two holes of 5 cm diameter for the hind limbs and two holes with a diameter of 4 cm for the forelimbs. The rat was placed on the platform by positioning first the hind limbs and then the forelimbs into the holes. The times taken by the animal to retract its first hind limb and the contralateral hind limb were recorded. The difference between the retraction times (sec) of both hind limbs was determined. This is considered to be an important parameter to measure functional abnormalities of the hind limbs, which are indicative for the extent of striatal degeneration [33]. The test was performed three times with a 45 min interval and the average values are reported.

2.6.4. String test for grip strength

The rat was allowed to hold with the forepaws a steel wire (2 mm in diameter and 35 cm in length), placed at a height of 50 cm over a cushion support. The length of time the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength [34].

2.7. Estimation of biochemical parameters

All the biochemical parameters were measured in the brain homogenate on day 15

2.7.1. Brain homogenate preparation

Animals were sacrificed by decapitation and brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with ice cold 0.1M phosphate buffer (pH 7.4) in a volume 10 times the weight of the tissue. The homogenate was centrifuged at 10,000×g for 15 min and aliquots of supernatant separated and used for biochemical estimation.

2.7.2. Protein estimation

Protein was measured in all brain samples by the method of Lowry [35] using bovine serum albumin (BSA) (1 mg/ml) as a standard.

2.7.3. Estimation of succinate dehydrogenase (SDH) activity

SDH is a marker of impaired mitochondrial metabolism in brain. The quantitative measurement of SDH levels in brain was performed according to the method as described in previous reports [36]. A 0.3 ml of sodium succinate solution was mixed with the 50 µl of gradient fraction of homogenate. The mixture was incubated at 37 °C for 10–20 min and then 0.1 ml of solution of p-iodonitrotetrazolium violet (INT) was added and again incubated for further 10 min. The reaction was stopped by adding the 1 ml of a mixture of ethyl acetate: ethanol: tricholoroacetic acid 5:5:1 (v/v/w) and centrifuged at 15,000 rpm for 1 min and the absorbance at 490 nm, determined with spectrophotometer (Shimadzu, UV-1700). Results were calculated using molar extinction coefficient of chromophore (1.36×10^4 M^{-1} cm^{-1}) and expressed as INT reduced µmol/mg protein.

2.7.4. Estimation of malondialdehyde (MDA)

The quantitative measurement of malondialdehyde (MDA) – end product of lipid peroxidation - in brain homogenate was performed according to the method of Wills [37]. The amount of MDA was measured after its reaction with thiobarbituric acid at 532nm using spectrophotometer (Shimadzu, UV-1700). The concentration of MDA was determined from a standard curve and expressed as nmol per mg protein.

2.7.5. Estimation of reduced glutathione (GSH)

Reduced glutathione in brain was estimated according to the method described by Ellman [38]. One ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1h. The samples were centrifuged at 1200×g for 15 min. To 1 ml of the supernatant, 2.7ml of phosphate buffer (0.1M, pH 8) and 0.2 ml of 5,5'dithiobis (2-nitrobenzoic acid) (DTNB) were added. The yellow color that developed was measured immediately at 412nm using a spectrophotometer. The concentration of glutathione in the supernatant was determined from a standard curve and expressed as µmol per mg protein.

2.7.6. Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green [39]. Equal volumes of supernatant and Greiss reagent were mixed, the mixture incubated for 10 min at room temperature in the dark and the absorbance determined at 540nm spectrophotometricaly. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as µMol/mg protein.

3. Results

3.1. Effect of vinpocetine on body weight in 3-NP treated rats.

Chronic, 3-NP treatment caused a significant decrease in body weight as compared to vehicle treated group [F (4, 29 = 212.9), $P < 0.05$] (Fig. 2). Pretreatment with vinpocetine (5, 10 and 20 mg/kg) in 3-NP treated rats significantly attenuated the 3-NP induced loss in body weight ($P < 0.05$). vinpocetine (10 mg/kg) treatment was

found to be most effective in curbing 3-NP induced.

3.2. Evaluation of Behavioral Parameters

3.2.1. Effect of vinpocetine on memory performance in Morris water maze task in 3-NP treated rats

Except 3-NP control, the latencies to reach the submerged platform decreased gradually in experimental animals of all other groups during 4 days of training in Morris water maze (MWM) task [Fig. 3]. On day 10^{th} there was no significant difference between the mean latencies of all groups [$F(4, 29) = 0.093$]. But the mean latencies were found to be significantly prolonged on day 11 [$F(4, 29) = 10.77$], 12 [$F(4, 29) = 23.32$] & 13 [$F(4, 29) = 50.43$], ($P < 0.05$) in the 3NP control as compared to vehicle control, indicating their inability to learn the task. But the 3-NP-induced acquisition deficit was significantly improved by chronic treatment (for 14 days) with vinpocetine (10 mg/kg). Between the treatment groups viz - vinpocetine (5, 10 and 20 mg/kg) used in the present study, the vinpocetine 10 mg/kg treatment was found to be most effective in ameliorating 3-NP induced spatial memory deficit. During the probe trial, with the platform removed, 3NP control rats failed to remember the precise location of the platform, spending significantly less time in the target quadrant compared to Vehicle control ($P < 0.05$). The mean time spent in the target quadrant by 3-NP administered groups treated with vinpocetine (5, 10 and 20 mg/kg) was significantly increased as compared to 3NP control group in a dose dependent manner, indicating improved consolidation of memory [$F(4, 29) = 74.53$, $P < 0.05$] [Fig. 4].

3.2.2. Effect of vinpocetine on spontaneous locomotor activity in 3-NP treated rats

The spontaneous locomotor activity on day 1 did not differ significantly among all the groups [$F(4, 29) = 0.39$, $P > 0.05$] (Fig. 5). However, 3-NP treatment caused a significant decrease in locomotor activity as compared to vehicle treated group ($P < 0.05$) as observed on day 8 and 15. Further, chronic treatment with vinpocetine (5, 10 and 20mg/kg) [$F(4, 29) = 71.81$, $F(4, 29) = 150$, $P < 0.05$ on day 8 and 15 respectively] significantly improved locomotor activity in 3-NP treated rats. However, vinpocetine (10 mg/kg) on Day 15 was found to be more effective compared to vinpocetine (5 mg/kg) ($P < 0.05$).

3.2.3. Effect of vinpocetine on Limb Withdrawal Test in 3-NP treated rats

In LWT (day 14), 3-NP treated group showed significant increase in the retraction time of the hind limbs as compared to the Vehicle control [$F(4, 29) = 65.3$, $P > 0.05$] (Fig. 6). Chronic treatment with vinpocetine (5, 10 and 20 mg/kg) significantly decreased the retraction time compared to 3-NP treated rats. However, no significant difference was observed between the vinpocetine 10 and 20 mg/kg.

3.2.4. Effect of vinpocetine on String test for grip strength in 3-NP treated rats

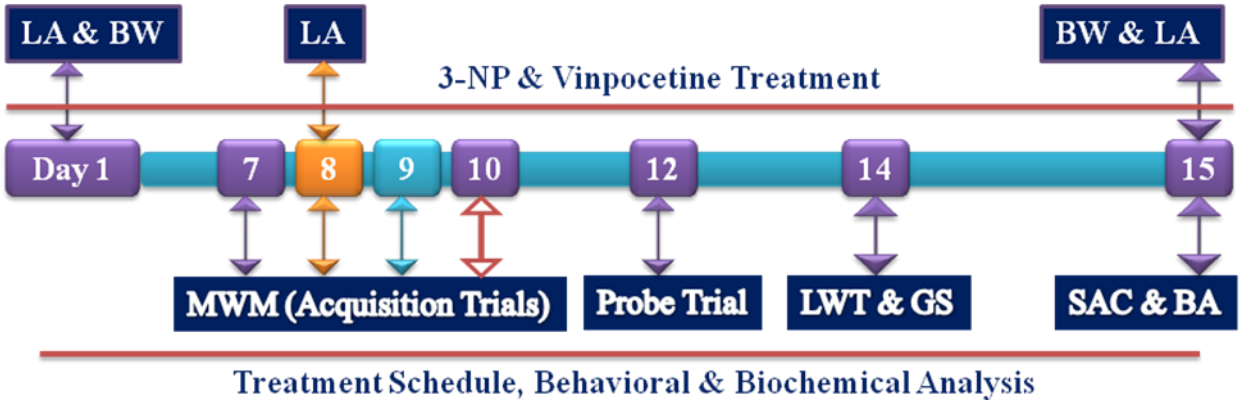
In 3-NP administered group a significant loss in grip strength was recorded, measured by the reduction in time to hold the metal wire as compared to Vehicle control [$F(4, 29) = 93.70$, $P < 0.05$] (Fig. 7), However, chronic treatment with vinpocetine (5, 10 and 20 mg/kg) significantly improved 3-NP induced loss in grip strength as compared to 3-NP control group in a dose dependent fashion.

3.3. EVALUATION OF BIOCHEMICAL PARAMETERS

3.3.1. Effect of vinpecetine on brain succinate dehydrogenase (SDH) activity in 3-NP treated rats

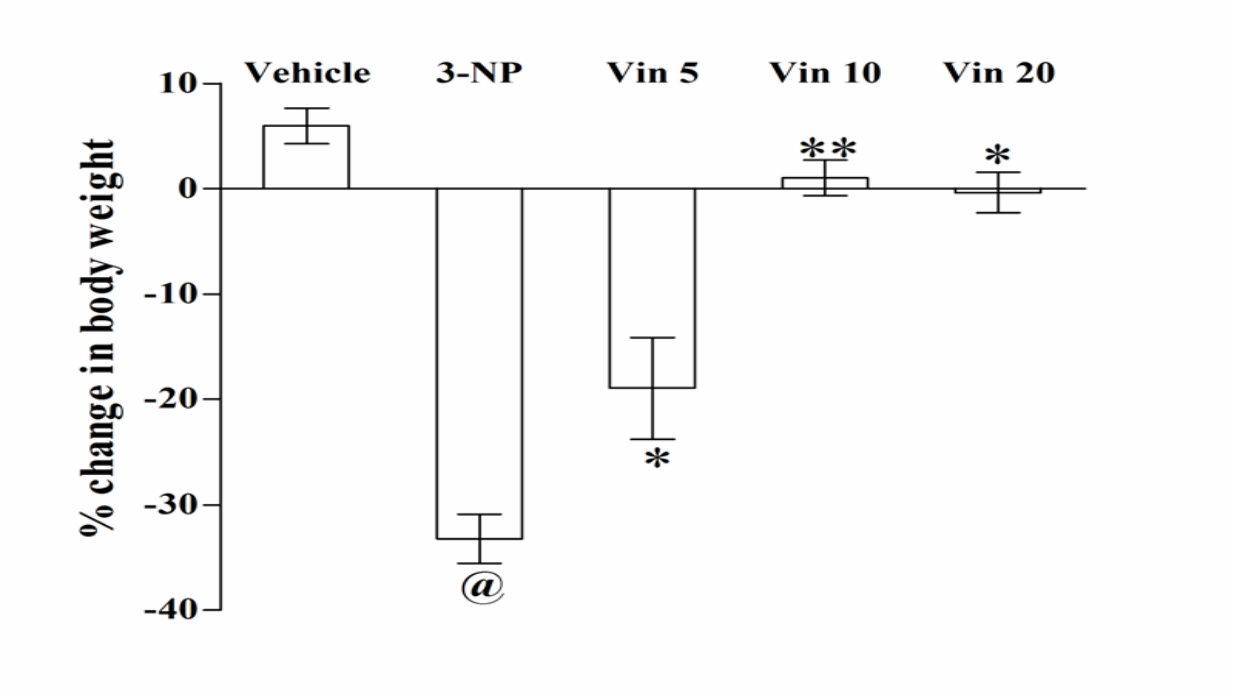
The activity of SDH was found to be decreased significantly in brain homogenate of 3-NP treated rats as compared with those of Vehicle control [$F(4, 29) = 54.61$, $P < 0.05$] (Fig. 8). Pretreatment with vinpocetine (5, 10 and 20 mg/kg) significantly and dose dependently restored the 3-NP induced decrease in SDH activity as compared to 3-NP control group ($P < 0.05$). Vinpocetine (10 mg/kg) was found to be more effective than vinpocetine 5 mg/kg.

Figure 1: Experimental Procedure ant treatment schedule



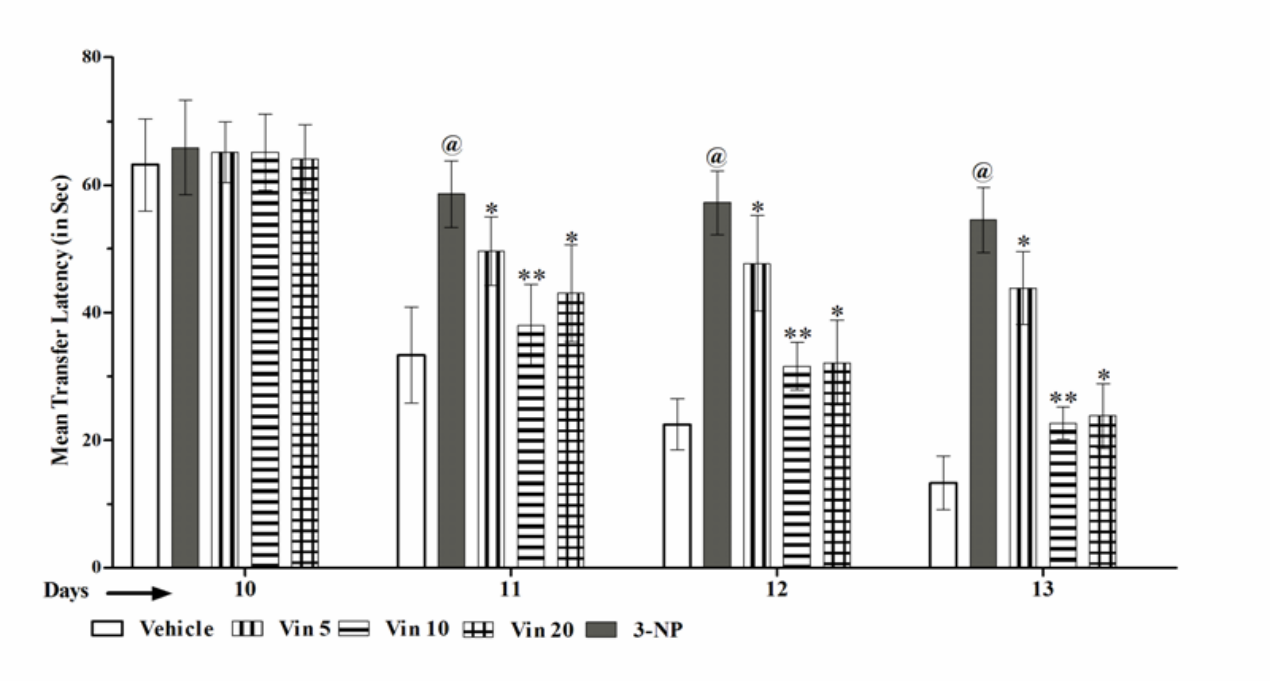
LA- Locomotor Activity, BW- Body Weight, MWM- Morris water maze, LWT- Limb Withdrawal Test, GS- Grip Strength, SAC- Sacrificed and BA- Biochemical Analysis

Figure 2: Effect of vinpocetine on body weight in 3-NP treated rats.



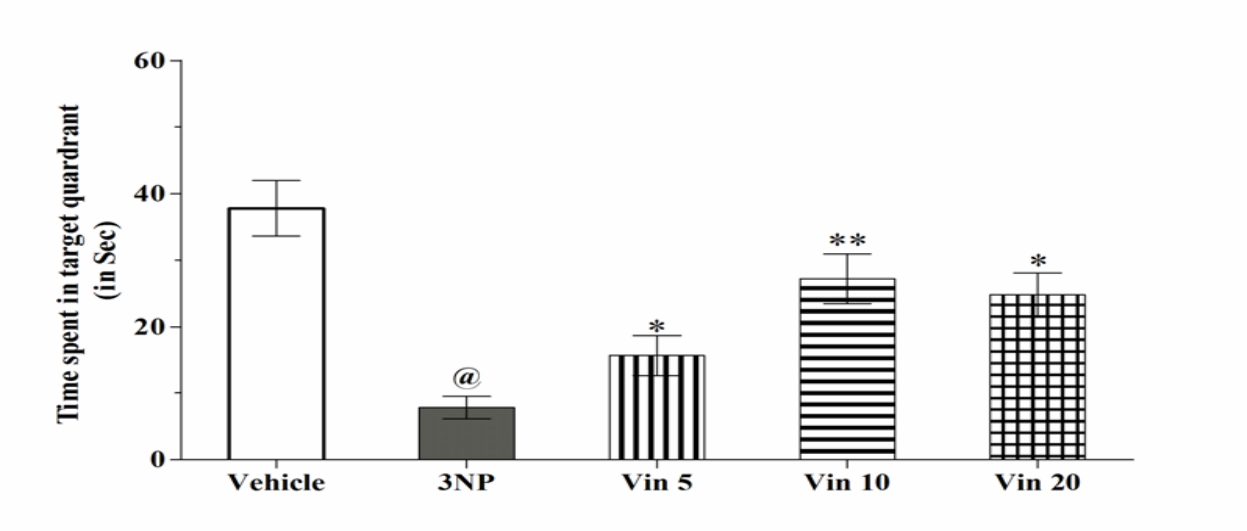
Values are expressed as mean ± SD. $^{@}P < 0.05$ as compared to vehicle, *P<0.05 versus 3-NP, **P<0.05 versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 3: Effect of vinpocetine on memory performance in Morris water maze task (Acquisition trial) in 3-NP treated rats.



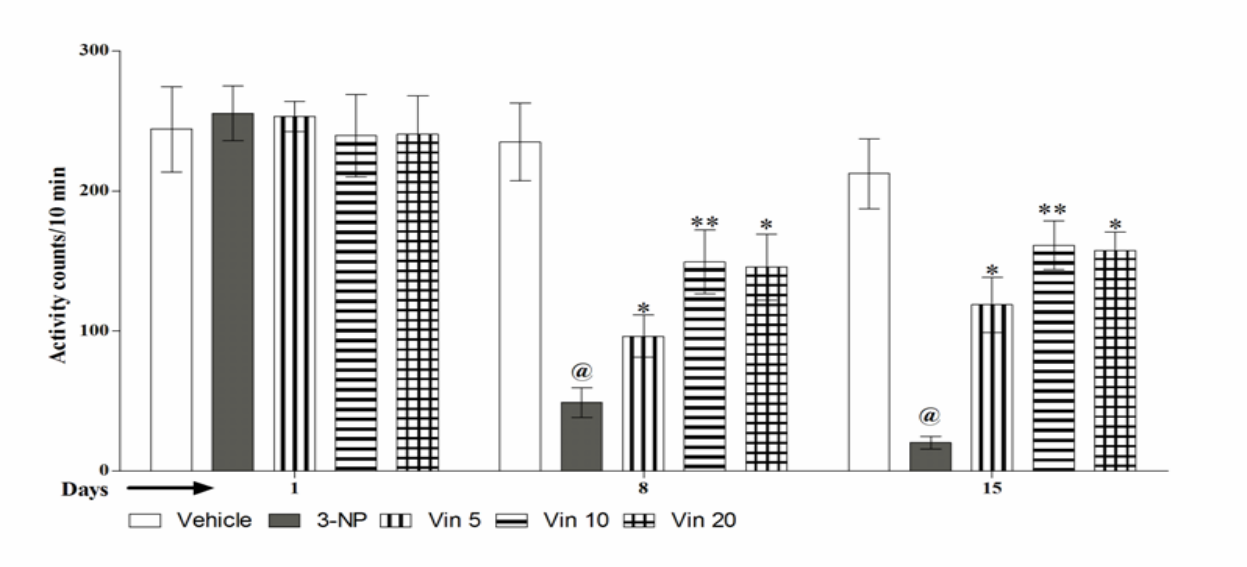
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 4: Effect of vinpocetine on memory consolidation in Morris water maze task (Retention trial) in 3-NP treated rats.



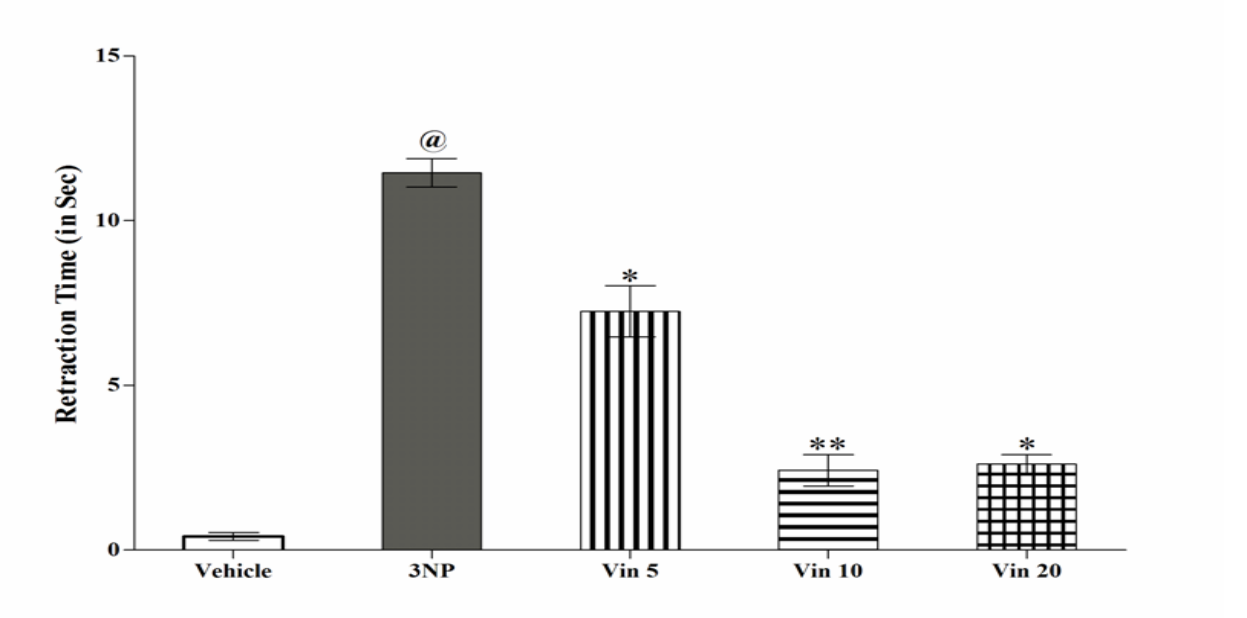
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, *$P<0.05$ versus 3-NP, **P<0.05 versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 5 - Effect of vinpocetine on spontaneous locomotor activity in 3-NP treated rats.



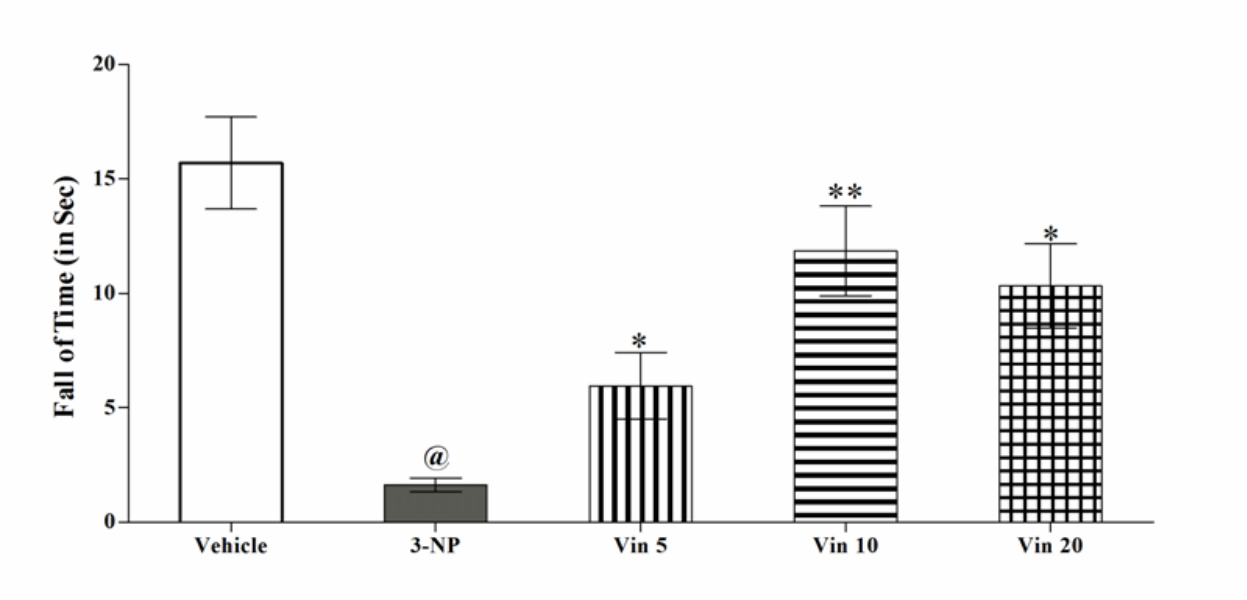
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 6: Effect of vinpocetine on retraction time in limb withdrawal test in 3-NP treated rats.



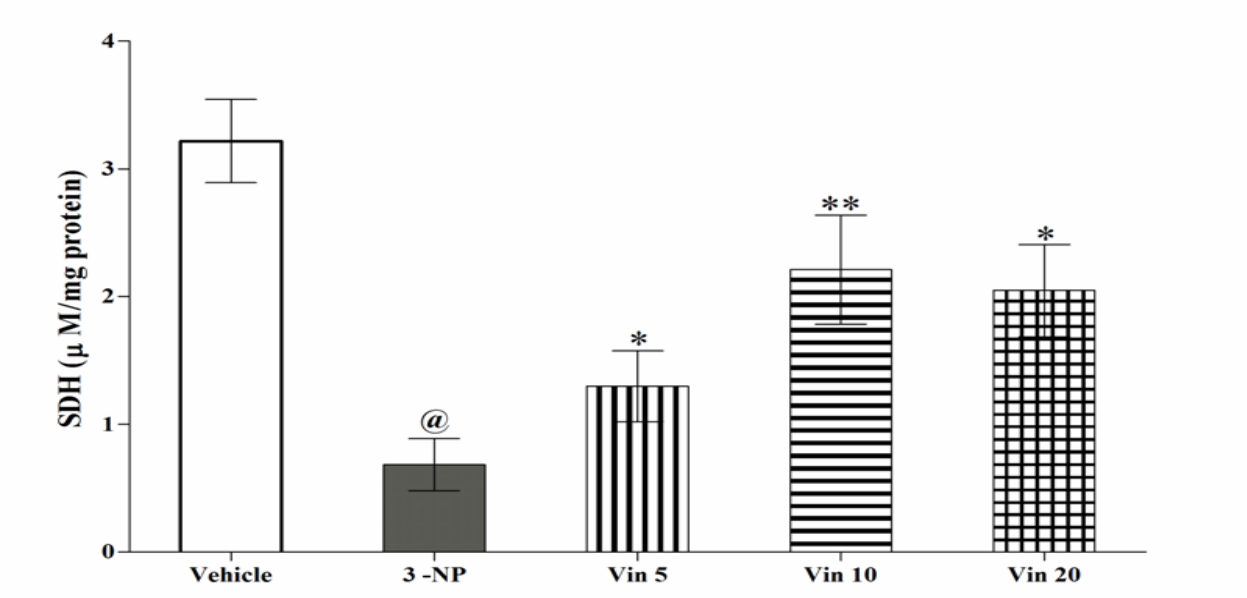
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of Vinpocetine (5 mg/kg).

Figure 7: Effect of vinpocetine on grip strength in 3-NP treated rats.



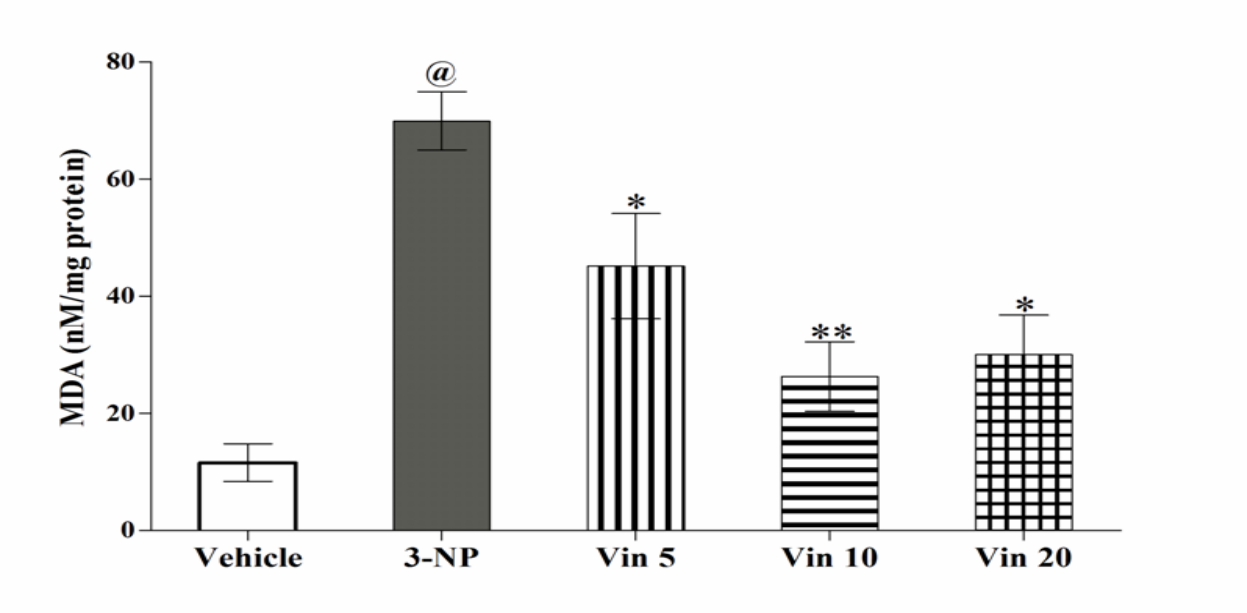
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 8: Effect of vinpocetine on striatal succinate dehydrogenase (SDH) activity in 3-NP treated rats.



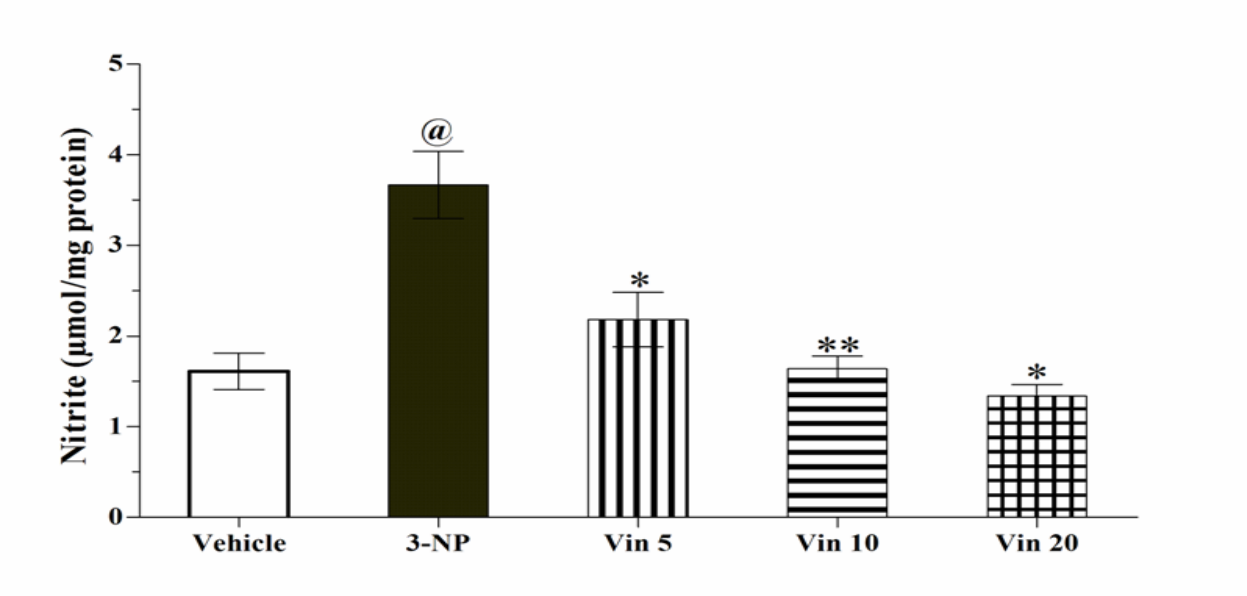
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 9: Effect of vinpocetine on striatal malondialdehyde (MDA) levels in 3-NP treated rats.



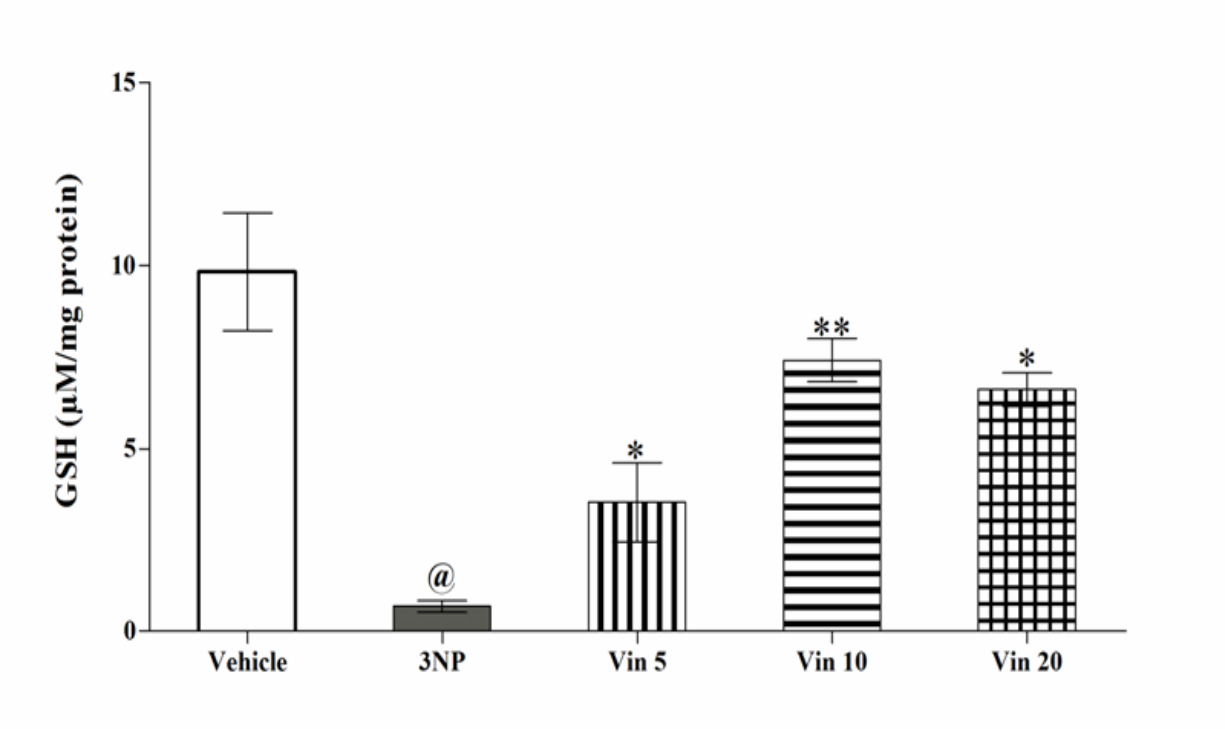
Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 10: Effect of vinpocetine on striatal nitrite levels in 3-NP treated rats.



Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

Figure 11: Effect of vinpocetine on striatal glutathione levels in 3-NP treated rats.



Values are expressed as mean ± SD. $^{@}P<0.05$ as compared to Vehicle, $^{*}P<0.05$ versus 3-NP, $^{**}P<0.05$ versus 3-NP and low dose of vinpocetine (5 mg/kg).

3.3.2. Effect of vinpocetine on brain malondialdehyde (MDA) levels in 3-NP treated rats

The level of MDA rose significantly in 3-NP control as compared to those of Vehicle treated rats ($P < 0.05$). But the treatment of these animals with vinpocetine (5, 10 and 20 mg/kg) significantly decreased MDA levels in a dose dependent manner as compared with those of 3-NP control [$F(4, 29) = 131$, $P < 0.05$] (Fig. 9).

3.3.3. Effect of vinpocetine on brain glutathione (GSH) levels in 3-NP treated rats

The levels of GSH were found to be significantly depleted after 14 days of 3-NP treatment as compared to Vehicle treated group animals ($P < 0.05$). Chronic treatment with vinpocetine (5, 10 and 20 mg/kg) was able to restore GSH levels in 3-NP treated rats [$F(4, 29) = 146.7$, $P < 0.05$] (Fig. 10).

3.3.4 Effect of vinpocetine on brain nitrite levels in 3-NP treated rats

The level of nitrite rose significantly following 14 days of 3-NP administration as compared to those of Vehicle control group ($P < 0.05$). However, these animals when treated chronically (for 14 days) with vinpocetine (5, 10 and 20 mg/kg) showed dose-dependent significant decrease in the nitrite levels, as compared with those of 3-NP control group [$F(4, 29) = 130.1$, $P < 0.05$] (Fig. 11).

Discussion

The present study demonstrates that vinpocetine-a PDE1 inhibitor, significantly attenuate 3-nitropropionic acid (3-NP) induced

cognitive and behavioral motor deficit and increased striatal oxidative- nutritive stress in rats. 3-NP is a mycotoxin and has been reported to inhibit succinate dehydrogenase (SDH) activity resulting in mitochondrial dysfunction and cellular energy deficits [40,41]. Reduction in body weight can be considered as an indicator of 3-NP induced neurotoxicity in rats. In the present study, chronic administration of 3-NP produced significant reduction in SDH activity and body weight in rats. Huntington's disease patients often show degeneration of hypothalamic neurons and loss of body weight [42]. Loss in body weight may be due to dysphagia as well as degeneration of hypothalamic orexin positive neurons [43,44]. Alternatively, loss in body weight and hypoactivity could be simply because of depressed energy metabolism after 3-NP administration. In the present study, vinpocetine dose dependently restored SDH activity and body weight in 3-NP treated rats, indicating its ability to restore cerebral energy. Indeed, vinpocetine has been reported to improve cerebral blood flow [45] perhaps by elevating cGMP levels and improve mitochondrial function [46], oxygen uptake and glucose utilization of whole brain [24,47] thereby restoring cerebral energy balance.

In the present study, chronic administration of 3-NP in rats produced significant acquisition and retention deficit in Morris water maze task. Further, 3-NP treatment also produced significant reduction in spontaneous locomotor activity, loss of grip strength and increase in retraction time in limb withdrawal tests, indicative of striatal degeneration and motor impairment [48]. The present findings are in tune with earlier reports, demonstrating similar cognitive and motor deficits following 3-NP administration in rats [49,50,51]. In the present study, chronic administration of vinpocetine in 3-NP treated rats, dose dependently improved cognitive and motor behaviors. Vinpocetine produced significant improvement in acquisition and retention in Morris water maze and restored motor functions such as locomotor activity, griping abilities and spontaneity of limb withdrawal in 3-NP treated rats. These results suggesting the ability of vinpocetine to restore striatal and hippocampal functions and may prevent degeneration of these neurons following 3-NP administration.

3-NP-induced cognitive and motor deficit could be related to its selective striatal and hippocampal neuronal damage [52-55]. Among the affected neurons in the hippocampal complex are the pyramidal neurons in the CA1 as well as various neurons in the CA3 region and dentate hilar area [55]. Besides, 3-NP has also been demonstrated to cause decrease in striatal cyclic nucleotide levels and its downstream CREB mediated transcriptional activity [31,32]. Indeed, similar decrease in cyclic nucleotide levels and CREB activity was observed clinically in HD patients [3-6,13,56,] as well as in genetic and pathogenic models of HD [10,11]. Decrease in cyclic nucleotides and CREB mediated transcriptional activity have been considered to play a major role in cognitive and motor abnormalities associated with HD pathology [8, 11]. Vinpocetine – a potent PDE1 inhibitor has been reported to induce significant rise in cAMP [23, 24] and cGMP levels [57,58] in rat brain. Moreover, vinpocetine has also been shown to increase CREB phosphorylation and improve memory retrieval in rats [25]. Thus, vinpocetine possibly by improving cerebral cyclic nucleotide levels may able restore CREB mediated transcriptional activity and attenuate 3-NP-induced cognitive and motor deficits in the present study. However, these mechanisms need to be investigated.

On the other hand, oxidative and nitrosative stress have also been implicated in the pahophysiology of HD [59] as well as in stiatal denegeneration following 3-NP administration in rats [60,61,62]. 3-NP has been reported to cause energy deficit which may contribute to excitotoxicity and increased oxidative stress [52,63]. In the present study, chronic administration of 3-NP produced significant decrease in striatal glutathione (GSH) levels and caused elevation in the malondialdehyde (MDA) and nitrite levels, indicating increased oxidative

and nitrosative stress. Our results are in line with earlier reports demonstrating increase in oxidative-nitrosative stress following 3-NP administration in rats [60,61,62]. MDA is an end product of lipid peroxidation and it was suggested that plasma MDA may be used as a potential biomarker to test treatment efficacy of drugs used in HD [64,65,66]. On the other hand glutathione (gamma-glutamyl-cysteinyl-glycine, GSH) is the most abundant intracellular antioxidant thiol which is central to redox defense during oxidative stress [67]. Therefore, decreased level of GSH may lead to the imbalance of the redox status in the cell, leading to oxidative stress. Furthermore, the role of glutathione in cognitive functions has also been documented [68]. Therefore, the maintenance of normal glutathione level is important for acquisition of spatial memory. On the other hand, glutathione unavailability has been reported to induce failure in hippocampal synaptic plasticity mechanisms, which could possibly be related to spatial memory deficit [69]. In the present study chronic administration of vinpocetine significantly and dose dependently attenuated 3-NP-induced increase in oxidative-nitrosative stress. Vinpocetine significantly decreased striatal MDA and nitrite and restored GSH levels in 3-NP treated rats in the present study. Vinpocetine has further been reported to improve cerebral blood flow [45], improve mitochondrial functions [46] and cerebral energy metabolism [24,70]. In addition, vinpocetine has also been demonstrated to have antioxidant potential and reported to scavenge hydroxyl radicals and inhibit lipid peroxidation [71,72,73].

In summary, the present study demonstrted that vinpocetine is effective in ameliorating 3-NP-induced cognitive and motor deficits and oxidative–nitritive stress. The observed beneficial effects of vinpocetine in cognitive and motor behaviors may be due to its antioxidant mechanisms. In addition, favorable modulation of cyclic nucleotide signaling by vinpocetine may also play a major role for the observed beneficial effects. Thorough verification of such properties might better clarify the mechanism of action of vinpocetine and support the rationale of clinical use of this PDE1 inhibitor in neurodegenerative disorders.

Acknowledgements

The authors express their gratitude to M/S Covex Pharma, Spain for providing ex-gratia sample of Vinpocetine. Authors are thankful to Mr. Parveen Garg, the chairman, ISF College of Pharmacy, Moga (Punjab) for valuable financial support and encouragement.

References

[1] Walker FO. Huntington's disease. Lancet 2007; 369: 218–228.

[2] Zuccato C, Valenza M, Cattaneo E. Molecular Mechanisms and Potential Therapeutical Targets in Huntington's Disease. Physiol Rev 2010;90:905–981

[3] Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc. Natl. Acad. Sci. U. S. A. 2000; 97:6763–6768.

[4] Steffan, JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, Kazantsev A, Schmidt E, Zhu YZ, Greenwald M, Kurokawa R, Housman DE, Jackson GR, Marsh JL, Thompson LM.. Histone deacetylase inhibitors arrest polyglutamine- dependent neurodegeneration in Drosophila. Nature 2001;413:739–743.

[5] Nucifora FC, Jr, Sasaki M, Peters MF, Huang H, Cooper JK. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science 2001; 291:2423–2428.

[6] Jiang H, Nucifora FC, Jr, Ross CA DeFranco DB. Cell death triggered by polyglutamine-expanded huntingtin in a neuronal cell line is associated with degradation of CREB-binding protein. Human Molecular Genetics 2003;12:1–12.

[7] Gil JM, Rego AC. Mechanisms of neurodegeneration in Huntington's disease. Eur. J. Neurosci 2008; 27:2803–2820

[8] Choi YS, Lee B, Cho HY, Reyes IB, Pu XA, Saido TC, Hoyt KR, Obrietan K. CREB is a key regulator of striatal vulnerability in chemical and genetic models of Huntington's disease. Neurobiol. Dis 2009; 36(2):259-68

[9] Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 2001; 293:493–498.

[10] Fusco FR, Zuccato C, Tartari M, Martorana A, De March Z, Giampà C, Cattaneo E, Bernardi G. Co-localization of brain-derived neurotrophic factor BDNF and wild-type huntingtin in normal and quinolinic acid-lesioned rat brain. Eur. J. Neurosci 2003;18:1093–1102.

[11] Cattaneo E, Zuccato C, Tartari M. Normal huntingtin function: an alternative approach to Huntington's disease. Nat. Rev. Neurosci 2005; 6:919–930

[12] Zuccato C, Cattaneo E. Role of brain-derived neurotrophic factor in Huntington's disease. Prog. Neurobiol 2007;81:294–330.

[13] Cramer H, Warter JM, Renaud B. Analysis of neurotransmitter metabolites and adenosine 3’,5'-monophosphate in the CSF of patients with extrapyramidal motor disorders. Advances in Neurology 1984;40(1):431– 435.

[14] Beavo JA. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev 1995; 75:725–748.

[15] Kakkar R, Raju RVS, Sharma RK. Calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE1). Cell. Mol. Life Sci 1999;55:1164–1186.

[16] Luthi-Carter R, Apostol BL, Dunah AW, DeJohn MM, Farrell LA, Bates GP, Young AB, Standaert DG, Thompson LM, Cha JH. Complex alteration of NMDA receptors in transgenic Huntington's disease mouse brain: analysis of mRNA and protein expression, plasma membrane association, interactingproteins, and phosphorylation. Neurobiological disorders 2004;14(3):624 – 636.

[17] Luthi-Carter R, Hanson SA, Strand AD, Bergstrom DA, Chun W, Peters NL, Woods AM, Chan EY, Kooperberg C, Krainc D, Young AB, Tapscott SJ, Olson JM. Dysregulation of gene expressionin the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Human Molecular Genetics 2002; 15(11):1911 – 1926.

[18] Desplats PA, Kass KE, Gilmartin T, Stanwood GD, Woodward EL, Head SR, Sutcliffe JG, Thomas EA. Selective deficits in the expression of striatal-enriched mRNAs in Huntington's disease. Journal of Neurochemistry 2006; 96(3):743 – 757.

[19] Crocker SF, Costain WJ, Robertson HA. DNA microarray analysis of striatal gene expression in symptomatic transgenic Huntington's mice (R6/2) reveals neuroinflammation and insulin associations. Brain Research 2006; 1088(1):176 – 186.

[20] Nguyen HP, Metzger S, Holzmann C, Koczan D, Thiesen HJ, von Hörsten S, Riess O, Bonin M . Age-dependent gene expression profile and protein expression in a transgenic rat model of Huntington's disease. Proteomics Clinical Applications 2008; 2(12):1638– 1650.

[21] Bonoczk Peter, Panczel Gyula, Nagy Zoltan. Vinpocetine increases cerebral blood flow and oxygenation in stroke patients: a near infrared spectroscopy and transcranial Doppler study.European journal of ultrasound 2002;15(1-2):85-91.

[22] Medina AE. Therapeutic utility of phosphodiesterase type-1 inhibitors in neurological conditions. Frontiers in neuroscience 2011; 5(21):1-5

[23] Rosdy B, Balázs M, Szporny L. Biochemical effects of ethyl apovincaminate. Arzneimittelforschung 1976; 26:1923-6.

[24] Kiss B, Lapis E, Palosi E, Groo D, Szporny L. Biochemical and pharmacological observations with vinpocetine, a cerebral oxygenator. In: Wauquier,A., Borgers, M.,Amery, W.K. (Eds.), Protection of tissues against hypoxia. Elsevier Biomedical Press,Amsterdam 1982;12:305-309.

[25] Krahe TE, Wang W, Medina AE. Phosphodiesterase inhibition increases CREB phosphorylation and restores orientation selectivity in a model of fetal alcohol spectrum disorders. PLoS One 2009;4:e6643.

[26] Marte A, Pepicelli O, Cavallero A, Raiteri M, Fedele E. In vivo effects of phosphodiesterase inhibition on basal cyclic guanosine monophosphate levels in the prefrontal cortex, hippocampus and cerebellum of freely moving rats. J Neurosci Res 2008;86:3338-47.

[27] Deshmukh R, Sharma V, Mehan S, Sharma N, Bedi K. L. Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine – a PDE1 inhibitor. Eur. J. Pharmacol 2009; 620:49–56.

[28] Zaitone SA, Elmatty DMA, Elshazly SM. Piracetam and vinpocetine ameliorate rotenone- induced Parkinsonism in rats. Indian journal of pharmacology 2012;44(6):774-779

[29] Blum D, Galas M.C, Gall D, Cuvelier L, Schiffmann, S.N. Striatal and cortical neurochemical changes induced by chronic metabolic compromise in the 3- nitropropionic model of Huntington's disease. Neurobiol. Dis. 2002;10, 410–426.

[30] Brouillet E, Jacquard C, Bizat N, Blum D. 3-Nitropropionic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease. J. Neurochem. 2005; 95, 1521–1540.

[31] Sugars KL, Rubinsztein DC. Transcriptional abnormalities in Huntington disease. Trends Genet 2003;19:233-8.

[32] Sugars KL, Brown R, Cook LJ, Swartz J, Rubinsztein DC. Decreased cAMP response element-mediated transcription: an early event in exon 1 and full-length cell models of Huntington's disease that contributes to polyglutamine pathogenesis. J Biol Chem 2004; 279:4988-99.

[33] Vis JC, Verbeek MM, De Waal RM, Ten Donkelaar HJ, Kremer HP . 3-Nitropropionic acid induces a spectrum of Huntington's disease-like neuropathology in rat striatum. Neuropathol Appl Neurobiol 1999; 25:513-21.

[34] Shear DA, Dong J, Gundy CD, Haik-Creguer KL, Dunbar GL. Comparison of intrastriatal injections of quinolinic acid and 3-nitropropionic acid for use in animal models of Huntington's disease. Prog. Neuropsychopharmacol Biol Psychiatry 1998; 22:1217-40.

[35] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

[36] Kumar P, Padi SSV, Naidu PS, Kumar A. Cyclooxygenase inhibition attenuates 3-nitropropionic acid-induced neurotoxicity in rats: possible antioxidant mechanisms. Fundam Clin Pharmacol 2007; 21:297-306.

[37] Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem J 1966; 99:667-76.

[38] Ellman GL. Tissue sulfhydryl groups. Arch. Biochem Biophys 1959; 82:70-7.

[39] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982; 126:131-8.

[40] Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller JM, Storey E, Srivastava R, Rosen BR, Hyman BT. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci 1993; 13:4181-92.

[41] Ming L. Moldy sugarcane poisoning--a case report with a brief review. J Toxicol Clin Toxicol 1995; 33:363-7.

[42] Borrell-Pages M, Zala D, Humbert S, Saudou F. Huntington's disease: from huntingtin function and dysfunction to therapeutic strategies. Cell Mol Life Sci 2006; 63:2642-60.

[43] Li SH, Yu ZX, Li CL, Nguyen HP, Zhou YX, Deng C, Li XJ. Lack of huntingtinassociated protein-1 causes neuronal death resembling hypothalamic degeneration in Huntington's disease. J Neurosci 2003; 23:6956-64.

[44] Petersen A, Gil J, Maat-Schieman ML, Bjorkqvist M, Tanila H, Araujo IM, Smith R,

Popovic N, Wierup N, Norlen P, Li JY, Roos RA, Sundler F, Mulder H, Brundin P. Orexin loss in Huntington's disease. Hum Mol Genet 2005;14:39-47.

[45] Imamoto T, Tanabe M, Shimamoto N, Kawazoe K, Hirata M. Cerebral circulatory and cardiac effects of vinpocetine and its metabolite, apovincaminic acid, in anesthetized dogs. Arzneimittelforschung 1984;34(2):161-9.

[46] Tarnok K, Kiss E, Luiten PG, Nyakas C, Tihanyi K, Schlett K, Eisel UL. Effects of Vinpocetine on mitochondrial function and neuroprotection in primary cortical neurons. Neurochem Int 2008;53:289-95.

[47] Shibota M, Kakihana M, Nagaoka A. The effect of vinpocetine on brain glucose uptake in mice. Nippon Yakurigaku Zasshi. Folia Pharmacol Jpn 1982;80:221-4.

[48] Vis JC, Verbeek MM, De Waal RM, Ten Donkelaar HJ, Kremer HP . 3-Nitropropionic acid induces a spectrum of Huntington's disease-like neuropathology in rat striatum. Neuropathol Appl Neurobiol 1999;25:513-21

[49] Keene CD, Rodrigues CM, Eich T, Linehan-Stieers C, Abt A, Kren BT, Steer CJ, Low WC. A bile acid protects against motor and cognitive deficits and reduces striatal degeneration in the 3-nitropropionic acid model of Huntington's disease. Exp Neurol 2001; 171:351-60

[50] Kumar P, Kumar A. Protective effect of rivastigmine against 3-NP induced HD like symptoms. European J Pharmacol 2009; 615:91-101

[51] Shear DA, Haik KL, Dunbar GL. Creatine reduces 3-nitropropionic-acid-induced cognitive and motor abnormalities in rats. Neuroreport. 2000;11:1833-7.

[52] Borlongan CV, Koutouzis TK, Freeman TB, Cahill DW, Sanberg PR. Behavioral pathology induced by repeated systemic injections of 3-nitropropionic acid mimics the motoric symptoms of Huntington's disease. Brain Res 1995;697:254-7.

[53] Duan W, Guo Z, Mattson MP. Participation of par-4 in the degeneration of striatal neurons induced by metabolic compromise with 3-nitropropionic acid. Exp Neurol 2000;165:1-11.

[54] Lee WT, Chang C. Magnetic resonance imaging and spectroscopy in assessing 3-nitropropionic acid-induced brain lesions: an animal model of Huntington's disease. Prog Neurobiol 2004;72:87-110.

[55] Miller PJ, Zaborszky L. 3-Nitropropionic acid neurotoxicity: visualization by silver staining and implications for use as an animal model of Huntington's disease. Exp Neurol 1997;146:212-29.

[56] Gines, S., Seong, IS., Fossale, E., Ivanova, E., Trettel, F., et al. Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. Human Molecular Genetics 2003; 12, 497–508.

[57] Staveren VWC, Markerink-van Ittersum M, Steinbusch HW, de Vente J. The effects of phosphodiesterase inhibition on cyclic GMP and cyclic AMP accumulation in the hippocampus of the rat. Brain Res 2001; 888:275-86.

[58] Marte A, Pepicelli O, Cavallero A, Raiteri M, Fedele E. In vivo effects of phosphodiesterase inhibition on basal cyclic guanosine monophosphate levels in the prefrontal cortex, hippocampus and cerebellum of freely moving rats. J Neurosci Res 2008; 86:3338-47.

[59] Kodsi MH, Swerdlow NR. Mitochondrial toxin 3-nitropropionic acid produces startle reflex abnormalities and striatal damage in rats that model some features of Huntington's disease. Neurosci Lett 1997;231:103-7.

[60] Borlongan CV, Kanning K, Poulos SG, Freeman TB, Cahill DW, Sanberg PR. Free radical damage and oxidative stress in Huntington's disease. J Fla Med Assoc 1996;83:335-41.

[61] French SJ, Humby T, Horner CH, Sofroniew MV, Rattray M. Hippocampal neurotrophin and trk receptor mRNA levels are altered by local administration of nicotine, carbachol and pilocarpine. Brain Res Mol Brain Res 1999;67:124-36.

[62] Haik KL, Shear DA, Schroeder U, Sabel BA, Dunbar GL. Quinolinic acid released from

polymeric brain implants causes behavioral and neuroanatomical alterations in a rodent model of Huntington's disease. Exp Neurol 2000;163:430-9.

[63] Tunez I, Montilla P, Muñoz MC, Drucker-Colín R. Effect of nicotine on 3-nitropropionic acid-induced oxidative stress in synaptosomes. Eur J Pharmacol 2004;504:169- 75.

[64] Chen CM, Wu YR, Cheng ML, Liu JL, Lee YM, Lee PW, Soong BW, Chiu DT. Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. Biochem Biophys Res Commun 2007;359:335-40.

[65] Dringen R. Metabolism and functions of glutathione in brain. Prog Neurobiol 2000;62:649-71.

[66] Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med 1990;8:583-99.

[67] Maher P. The effects of stress and aging on glutathione metabolism. Ageing Res Rev 2005;4:288-314.

[68] Cruz R, Almaguer Melian W, Bergado Rosado JA. Glutathione in cognitive function and neurodegeneration. Rev Neurol. 2003;36:877-86.

[69] Cruz-Aguado R, Almaguer-Melian W, Díaz CM, Lorigados L, Bergado J. Behavioral and biochemical effects of glutathione depletion in the rat brain. Brain Res Bull 2001;55:327-33.

[70] Shibota M, Kakihana M, Nagaoka A. The effect of vinpocetine on brain glucose uptake in mice. Nippon Yakurigaku Zasshi. Folia Pharmacol Jpn 1982;80:221-4.

[71] Stolc S. Indole derivatives as neuroprotectants. Life Sci 1999;65:1943-50.

[72] Santos MS, Duarte AI, Moreira PI, Oliveira CR. Synaptosomal response to oxidative stress: effect of vinpocetine. Free Radic Res 2000;32:57-66.

[73] Pereira C, Agostinho P, Moreira PI, Duarte AI, Santos MS, Oliveira CR. Neuroprotection strategies: effect of vinpocetine in vitro oxidative stress models. Acta Med Port 2003;16:401