Research article **Bimonthly published scientific journal**

INTERNATIONAL JOURNAL of Indigenous Herbs and Drugs

ISSN-2456-7345

Neuroprotective effects of ascorbic acid against 3-NP induced Huntington's disease in experimental rats

Dharmender Jaglan, Chanpreet Singh

Chandigarh College of Pharmacy, Landran, Mohali, Punjab, India

Abstract

Objective: The present study was designed to evaluate the neuroprotective effects of ascorbic acid against 3-nitropropionic acid (3-NP) induced huntington's disease [HD] in rats and possible involvement of $GABA_A$ receptors.

Methods: 3-nitropropionic acid (10 mg/kg) was administered intraperitoneally (i.p) once daily for a period of 14 days to induce the symptoms of HD. In this study bicuculline was employed as an antagonist to confirm the involvement of GABA receptor. All the behavioural parameters were observed before drug administration and 24 hrs after first dose and 24 hrs after the last dose, that on the 15^{th} day after the start of 3-NP treatment.

Results: Administration of ascorbic acid (200 mg/kg, i.p.) per se had no effect on acquisition, memory, motor activity and various biochemical parameters as compared to control group animals. Pre-treatment with ascorbic acid (100 mg/kg and 200 mg/kg, i.p.) once daily for a period of 14 days attenuated 3-NP induced motor and cognitive impairement together with improvement in biochemical parameters (↑ GSH, ↓ MDA, ↓ AChE, ↓ MPO& ↓ iNOS) in a dose dependent manner in rats. Pre-treatment with bicuculline (1 mg/g, i.p.) once daily for a period of 14 days abolished the neuroprotective effects of ascorbic acid on 3-NP treated rats. In addition, histopathological changes in present study have further justified the approach.

Conclusion: The results of the present study demonstrate that ascorbic acid has shown neuroprotective effects against 3-NP induced behavioural and biochemical alterations similar to Huntington's disease in rats and the said role of ascorbic acid involves the activation of $GABA_A$ receptors..

Keywords: Ascorbic Acid, Huntington's Disease, Neuroprotective Effect, 3-Nitropropionic Acid

Introduction

Huntington's disease (HD) is a chronic progressive autosomal dominant neurodegenerative disorder that is characterized by a striatal-specific degeneration [1]. The pathological changes manifest clinically in midlife as a triad of cognitive decline, psychiatric disturbance and impairment of motor function. The most recent model of HD is based on systemic injections of 3-nitropropionic acid (3-NP), a mitochondrial toxin that causes striatal neuropathy similar to that seen in clinical HD [2, 3]. Ascorbic acid is an important antioxidant with multiple cellular functions. It plays a role in detoxification processes, participates as an enzymatic cofactor modulates synaptic activity and neuronal metabolism, among other functions. Ascorbic acid is concentrated in the brain. The brain is responsible for the 25% of total body glucose utilization. Such elevated activity correlates with a high oxidative metabolism and therefore the brain is dependent on antioxidants for protection against pathological conditions. Redox imbalance and oxidative stress are observed during aging and in neurodegenerative disorders such as alzheimer's disease (AD), parkinson's disease (PD), huntington's disease (HD), amyotrophic lateral sclerosis (ALS), among others. Imbalance of ascorbic acid homeostasis has also been demonstrated in neurodegenerative disorders [4]. There has been a lot of research at various levels involving $GABA_A$ receptors in HD. But, direct involvement of ascorbic acid in HD and its action through $GABA_A$ receptor has not been studied. So, present study was designed to investigate the activation of $GABA_A$ receptor as potential molecular mechanism in ascorbic acid-mediated protection against 3-NP induced HD. The animal model used in the present study is a well proven model which employs administration of 3-NP (3-Nitropropionic acid) to induce pathophysiology similar to Huntington's disease in rats. Behavioural paradigms and biochemical markers was tested to access the extent of neurodegneration induced by 3-NP protection provided by ascorbic acid.

Materials and methods

Drugs and chemicals

All chemicals and biochemical reagents of analytical grade and highest purity were used. The following agents were used in the present study: 3-nitropropionic acid (Sigma-Aldrich Corporation, India) was diluted with saline (adjusted pH 7.4

Jaglan D, Singh C. Neuroprotective effects of ascorbic acid against 3-NP induced Huntington's disease in experimental rats. Int J Ind Herbs Drugs 2017; 2(6): 1-10.

with NaOH) and administered intraperitoneally (i.p), Ascorbic Acid (LobaChemiePvt.LTD.), bicuculline (Cayman chemicals company) was used as antagonist of GABA receptor and DMSO was used to dissolve ascorbic acid.

Experimental animals

Male wistar rats (procured from National Institute of Pharmaceutical Education and Research, Mohali) maintained in the central animal house of the facility of Chandigarh College of Pharmacy, Landran, Mohali, Punjab and weighing between 200 and 250 g of weight were used. The animals were kept under standard conditions of light and dark cycle with food and water *ad libitum* in plastic cage with husk bedding. All the experiments were carried out between 09:00 and 15:00 hr in semi- sound proof laboratory conditions. The protocol was approved by the Institutional Animal Ethics Committee vide no (1201/a/08/CPCSEA) and carried out in accordance with the Indian National Academy Guidelines for the use and care of animals. Adequate measures were taken to minimize the pain and discomfort to the animals. The care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

Experimental protocol

Animal model for Huntington's disease

3-nitropropionic acid (10 mg/kg) was administered intraperitoneally (i.p) once daily for a period of 14 days to induce the symptoms of HD. 3-NP injection was freshly prepared in normal saline (0.5 ml of normal saline per 100gm of animal weight) before administration. All animals were acclimatized for at least 2 hrs before testing unless otherwise specified in all the experiments. Animals were divided in seven groups and each group consisted of six animals as approved by IAEC for the post emptive paradigms. To evaluate the effect of ascorbic acid (100, 200 mg/kg, i.p) treatment was initiated on the 1st day and continued till 14th day and 3-nitropropionic acid was also administered along with ascorbic acid.

Group I (Control, n= 6)

Rats were administered with normal saline (10 mg/kg/day, i.p) 30 min before the acquisition trails conducted from day 1 to day 4 and 30 min before retrieval trail conducted on day 5 using Morris water maze (MWM) test.

Group II (DMSO control, n= 6)

Rats were administered with MMSO (1 mg/kg/day, i.p) daily for 14 days followed by exposure to MWM test.

Group III (3- NP control, n= 6)

The rats were administered with 3- nitro-proponic acid (3- NP) dose (10 mg/kg/day, i.p, 0.9 % w/v, i.p) for 14 days followed by the exposure to the MWM test.

Group IV (ascorbic acid *per se*, n= 6)

Rats were administered with ascorbic acid (200 mg/kg/day, i.p), 30 min before acquisition trails from day 1 to day 4

Group V (3-NP + ascorbic acid (LD), n=6)

Ascorbic acid (100 mg/kg/day, i.p) was administered to rats 30 min before 3-NP (10 mg/kg/day, i.p) administration.

Group VI (3- NP + ascorbic acid (HD), n=6)

Ascorbic acid (200 mg/kg/day, i.p) was administered to rats 30 min before 3-NP (10 mg/kg/day, i.p) administration.

Group VII (bicuculline + ascorbic acid (HD) + 3-NP, n=6)

Bicuculline (10 mg/kg/day,i.p) was administered to rats 30 min before administration of ascorbic acid. Ascorbic acid (200 mg/kg/day, i.p) was administered 30 min before 3-NP.

Only rats with positive behavioral effects were included in the study. All animals were trained for 4 days before administration. All the behavioral parameters were observed before drug administration and 24 hrs after first dose and 24 hrs after the last dose, that on the 15th day after the start of 3-NP treatment [5].

Measurement of body weight

Body weight was noted on the first and last days of the experiment. Percentage change in body weight was calculated in comparison with initial body weight on the first day of experimentation.

Behavioural assessments

Elevated plus maze paradigm

Elevated plus maze is used to test learning and memory. The elevated plus maze consisted of two opposite open arms crossed with two closed arms of the same dimensions. The arms are connected with a central square. Acquisition of memory was assessed on the day 1st before initiating 3-NP treatment in control group. Rats were placed individually at one end of an open arm facing away from the central square. The time taken by the animal to move from the open arm into the closed arm is recorded as the initial transfer latency. The animals were allowed to explore the maze for 30 sec after recording the initial latency and then returned to its home cage. If animal did not enter an enclosed arm within 90 sec, it was gently pushed in to the enclosed arm and the transfer latency is assigned as 90 sec. Retention of memory was assessed by similarly placing a rat on an open arm and noting the retention latency 24 hr (day 2) and 4 days (day 5) after the initial transfer latency (ITL). These times were referred to as the first retention transfer latency and second retention transfer latency, respectively [6, 7].

Morris water maze (Spatial navigation task)

The acquisition and retention of the spatial task was examined using the Morris water maze. Animals were trained to swim to a platform in a circular pool located in a test room. The pool was filled with water (24+ 2c) to a depth of 40 cm. A movable circular platform, 9 cm in diameter and mounted on a column was placed in the pool 1 cm above the water level (visible platform) for the maze acquisition test. Another movable platform, 9 cm in diameter and mounted on a

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

column, was placed in the pool 1 cm below the water level (hidden platform) for the maze retention test.

Maze acquisition test (training)

Animals received a training session consisting of 4 trails in day for four days before 3-NP administration. In all 4 days, the starting positions were different. The latency to find the escape platform was recorded up to a maximum of 2 min. The visible platform was fixed in the centre of one of the 4 quadrants and remained there throughout the experiment. The time taken by a rat to reach the platform on the fourth day was recorded as the initial acquisition latency.

Maze retention test (testing for retention of the learned task)

Following 24 h (day 5) and 14 days (day 15) after the initial acquisition latency (IAL), rats were randomly released at any edges facing the wall of the pool and tested for the retention of the response. The time taken to reach the hidden platform on days 5 and 15 following initiation of 3-NP treatment was recorded and termed as the first retention latency and second retention latency, respectively.

Assessment of gross locomotor activity by using actophotometer

The locomotor activity was monitored using actophotometer on 1st, 5th, 15th, day of 3-NP administered and ascorbic acid treated groups [6]. The horizontal motor activity was detected by two perpendicular arrays of 15 infrared beams located 2.5 cm above the floor of the testing area. Each interruption of a beam on the x or y-axis generated an electric impulse, which was presented on a digital counter. Similarly, the vertical motor activity was recorded using the additional row of infrared sensors located 12 cm above the floor. Each animal was observed over a period of 5 min and values expressed as counts per 5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room [8].

Assessment of grip strength activity by using rotarod

All animals were evaluated for motor ability and balance by using the rotarod on 1st, 5th, and 15th day of treatment [6]. The rats were given a prior training session before initialization of any therapy to acclimate them to rotarod apparatus. Rats were placed on the rotating rod with a diameter of 7 cm (speed 25 rpm). The length of time in the rod was taken as the measure of competency. The cutoff time as 180 sec and each rat performed three separate trials. The average result was recorded [6-8].

Assessment of the biochemical parameters

Biochemical tests were carried out after the last behavioural test.

Tissue preparation

Animals were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized (10 times (w/v) with ice – cold 0.1M phosphate buffer (pH 7.4). the homogenate was centrifuged at 10,000 x g for 15 min and aliquots of supernatant were separated and used for biochemical experiments.

Thiobarbituric acid reactive substances (TBARS)

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in renal tissue was performed according to method of Nichans and Samuelson, (1968) [9].

Reduced glutathione (GSH) level

The whole brain level was estimated by method of Beutler et al, (1963) [10].

Estimation of total protein content

Brain total protein was estimated by the method of Lowry et al. (1951) with slight modifications using bovine serum albumin (BSA) as a standard [11].

Estimation of brain nitrite / nitrate concentration level

The accumulation of nitrite in the supernatant was measured as an indicator of the production of nitric oxide determined by the method of Green et al.(1982) using a colorimetric assay [12].

Estimation of brain acetyl cholinesterase (AChE) activity

The cholinergic neuron marker acetylcholinesterase, was estimated in whole brain according to the method of Ellman et al. (1961) with slight modifications (Koladiya et al., 2009) [13, 14].

Myeloperoxidase activity

The myeloperoxidase (MPO) activity which is measured as an index of neutrophil accumulation which can be measured by using method of Krawisz et al., (1984) [15].

Histological evaluation

Haematoxylin and eosin staining of brain tissue was used for histological parameters. The brain tissues was preserved in 10% formalin and dehydrated in graded concentrations of ethanol, immersed in xylene and then embedded in paraffin. The sections of 4 µm thickness was cut and placed on slide using commercial Baker's mounting fluid. Paraffin wax was removed by warming the slide gently, until the wax melts and then it was washed with xylene. This was followed by washings with absolute alcohol and water to hydrate the sections and stained with haematoxylin and eosin described by Clayden (1971) [16]. The hydrated sections were stained with haematoxylin for 15 min. The stained sections were washed with water and it was treated with 1% acid alcohol mixture for 20 s. The acid alcohol mixture was washed off with water and sections were counterstained with 1% aqueous solution of eosin for 2 min. After washing with water to remove excess of eosin, the sections was dehydrated using absolute alcohol and then mounted using Canada balsam as mounting agent. The slides were observed for gross histopathological changes and neutrophil accumulation.

Results and discussion

There was no change in the initial and final body weight of

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

control animals. However, 3-NP treatment caused a significant decrease in body weight on day 15th as compared to control group. Ascorbic acid *per se* (200 mg/kg, *i.p*) and vehicle treatment had no effect on body weight, however pre-treatment with ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) on 3-NP treated rats significantly and dose dependently prevented the decrease in the body weight (Table 1). Moreover treatment with bicuculline (1 mg/kg, *i.p*) significantly abolished the effects of ascorbic acid.

Table 1: **Effects of various interventions on body weight of animals**

Groups	Treatment	Initial body weight(g)	Final body weight(g)
1	Control	222.5±6.5	219±7.4
2	DMSO	220.6±9.2	218.3±8.3
3	3-NP[a]	221.6±8.8	208.0±8.4
4	Ascorbic acid	220.0±10.4	218.3±6.5
5	LD + 3-NP[b]	220.6±9.7	213.6±7.3
6	HD + 3-NP[b]	220.0±7.7	217.3±7.6
7	Bicuc + ascorbic acid + 3-NP[c]	221.6±8.5	210.3±6.0

Values are the Mean ± S.E.M; a= p<0.05 as compared to the normal control; b= p< 0.05 as compared to the 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v,*i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = Low dose ascorbic acid (100 mg/kg, *i.p*); HD = High dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*)

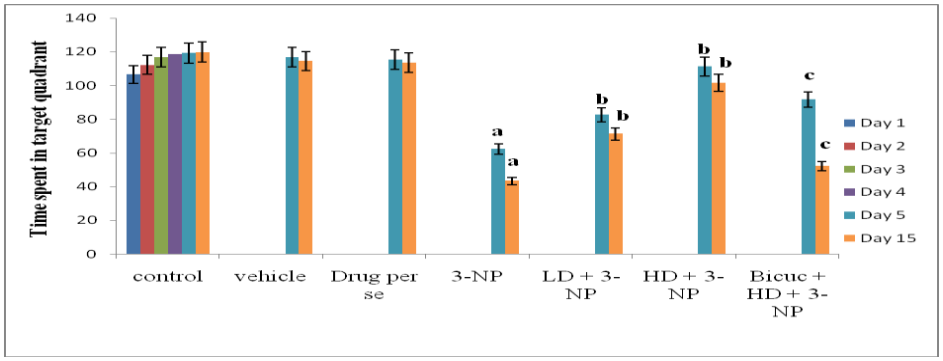

Figure 1: Effects of ascorbic acid on memory and learning using Morris Water Maze test

Values are the mean ± SEM; a= p<0.05 as compared to the normal control; b= p< 0.05 as compared to the 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); 5th day F= 8.982, DF =6, 35; 15th day F= 8.541, DF=6, 35

All the animals showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task. The mean retention latencies to escape onto the hidden platform did not alter in control rats on all days throughout the experiment. However, the mean retention latencies in the 3-NP (10 mg/kg, i.p) treated rats were increased significantly (p<0.05) after initial training in the water maze on days 5thand 15th of 3-NP as compared to control group. The time spent in the target quadrant (TSTQ) was also decreased in the 3-NP treated rats as compared to control group. Ascorbic acid (200 mg/kg i.p.) *per se* and vehicle treatment had no effect on memory and learning when compared with control group. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) abolished the ascorbic acid mediated reduction in retention latencies (Figure 1).

In this study, the mean initial transfer latencies (ITL) on day 1 before 3-NP treatment for each rat was relatively stable and showed no significant variation. All the rats entered the closed arm within 96 sec. The vehicle and control treated rats entered the closed arm quickly, and the mean retention transfer latencies (1st RTL and 2nd RTL) to enter the closed arm on days 5 and 15 were shorter as compared to the ITL on day 1 of each group. In contrast, the 3-NP (10 mg/kg, i.p) treated rats performed poorly throughout the experiment and an increase in the mean retention transfer latencies on day 5 and 15 was noted as compared to the pre-training latency on day 1, demonstrating 3-NP induced cognitive dysfunction. Ascorbic acid administration (100 mg/kg and 200 mg/kg, *i.p*) to 3-NP (10 mg/kg, i.p) treated rats significantly decreased the mean retention latencies on day 5 and day 15 indicating

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

an improvement in memory impairement induced by 3-NP. However, ascorbic acid (200 mg/kg i.p.) *per se* treatment had no effect on memory when compared with control group. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) abolished the effect of ascorbic acid (Figure 2).

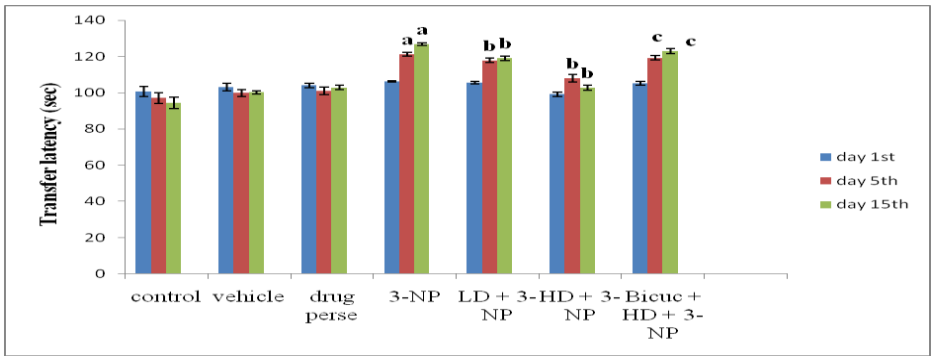

Figure 2: Effects of ascorbic acid on memory performance in elevated plus maze test

Values are the mean ± SEM; a= p<0.05 as compared to the normal control ; b= p< as compared to the 3NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); 1st Day F= 21.64, DF= 6, 35; 5th Day F= 18.393, DF= 6, 35; 15th Day F= 25.320, DF= 6, 35.

3-NP (10 mg/kg, i.p) treatment impaired grip strength as assessed by rotarod test on 5th day and 15th day. Pre-treatment with ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) significantly (P<0.05) attenuated the 3-NP induced decrease in grip strength in a dose dependent manner on day 5 and 15. However, ascorbic acid *per se* (200 mg/kg, *i.p*) and vehicle treatment had no effect on grip strength activity when compared with control group. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) significantly abolished the ascorbic acid mediated increase in grip strength (figure 3).

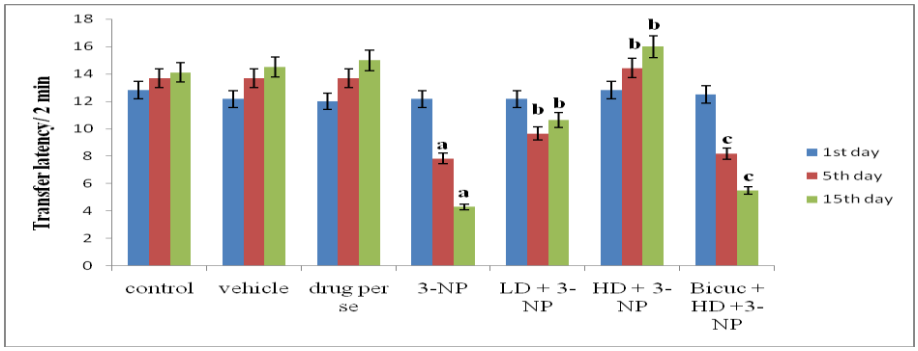

Figure 3: Effects of ascorbic acid on grip strength using rotarod test

Values are the mean ± SEM; a= p< 0.05 as compared to the normal control; b= p< 0.05 as compared to the 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); Ist Day F= 0.1475, DF= 6, 35; 5th Day F= 35.236, DF= 6, 34; 15th Day F= 58.245, DF= 6, 34.

3-NP administration caused significant decrease in the locomotor activity on day 5 and day 15 as compared to control group. Pre-treatment with ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) significantly (P<0.05) attenuated the 3-NP (10 mg/kg, i.p) induced decrease in motor activity in a dose dependent manner on day 5 and day 15 and there was no change in the locomotor activity of vehicle treated rats as compared to control group. However, ascorbic acid *per se* (200 mg/kg, *i.p.*) and vehicle treatment had no effect on gross behavioral activity when compared with control group. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) significantly abolished the ascorbic acid mediated increase in motor activity (figure 4).

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

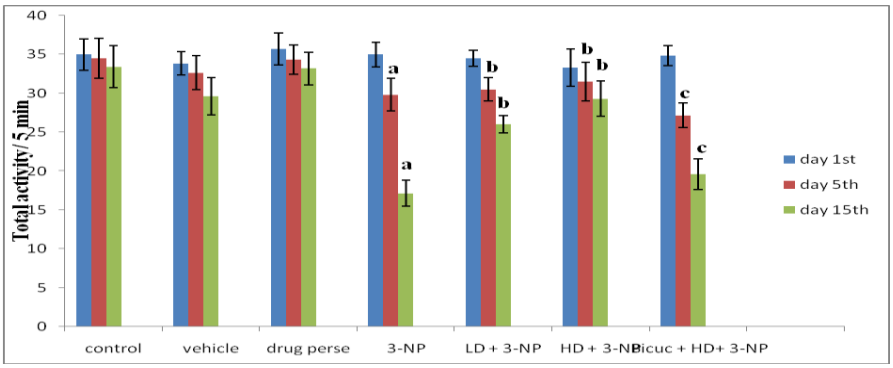

Figure 4: Effects of ascorbic acid on locomotor activity using actophotometer

Values are the mean ± SEM; a= p<0.05 as compared to the normal control; b= p< 0.05 as compared to the 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); Ist Day F= 970.12, DF= 6,35; 5th Day F= 2.259, DF= 6, 35; 15th Day F= 6.023, DF= 6, 35.

GSH levels in 3-NP (10 mg/kg, i.p) administered rats have shown significant (P<0.05) decrease as compared with control group whereas, there was no effect of ascorbic acid (200 mg/kg, *i.p*) *per se* and vehicle treatment on brain GSH levels. Pre-treatment with ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) significantly (P<0.05) attenuated 3-NP induced decrease in GSH levels. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) abolished the ascorbic acid mediated increase in brain GSH levels (Figure 5).

Systemic administration of 3-NP (10 mg/kg, i.p) caused a marked increase in lipid peroxidation in addition to a decline in antioxidant defense, as indicated by a significant (P<0.05) rise in brain MDA levels as compared to the control rats. Further, there were no alterations in the brain MDA level due to ascorbic acid (200 mg/kg, *i.p*) *per se* and vehicle treatment as compared to control group. Pretreatment with ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) significantly (P<0.05) prevented the increase in MDA levels, with marked effect observed at the highest dose when compared to the 3-NP treated group. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) abolished the protective effects of ascorbic acid (Figure 6).

In present study there was no significant effect of ascorbic acid *per se* (200 mg/kg, *i.p*) and vehicle treatment on brain nitrite levels as compared to control rats. Systemic 3-NP (10 mg/kg, i.p) administration caused a significant (P<0.05) increase in brain nitrite levels, which was significantly (P<0.05) prevented by pretreatment with ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) in a dose dependent manner. Moreover, treatment with bicuculline (1mg/kg, *i.p*) abolished the ascorbic acid mediated reduction in brain nitrite levels (Figure 7).

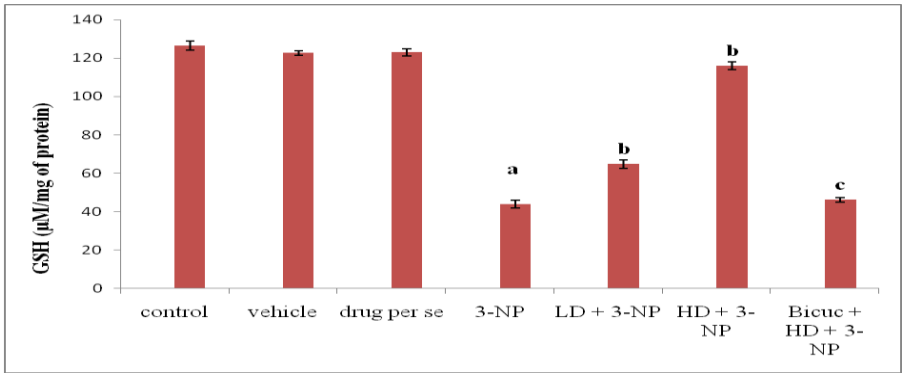

Figure 5: Effects of ascorbic acid on reduced glutathione levels

Values are the mean ± SEM; a= p< 0.05 as compared to the normal control; b= p< 0.05 as compared to 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); F= 477.38; DF= 6, 35.

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

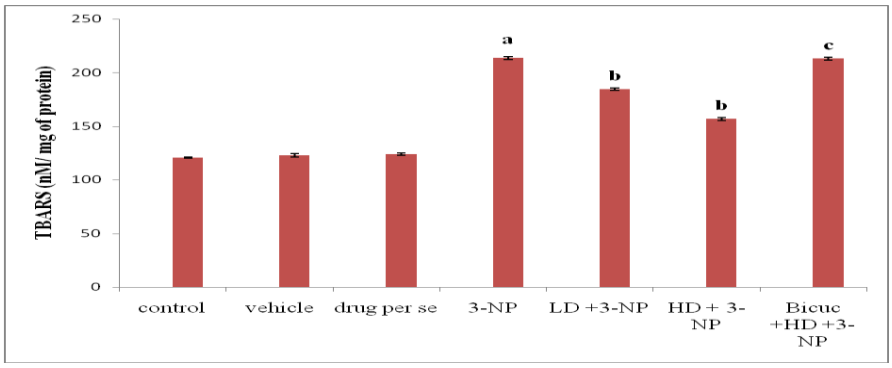

Figure 6: Effects of ascorbic acid on lipid peroxidation

Values are the mean ± SEM; a= p< 0.05 as compared to the normal control; b= p< 0.05 as compared to 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); F= 1191.7; DF= 6, 35.

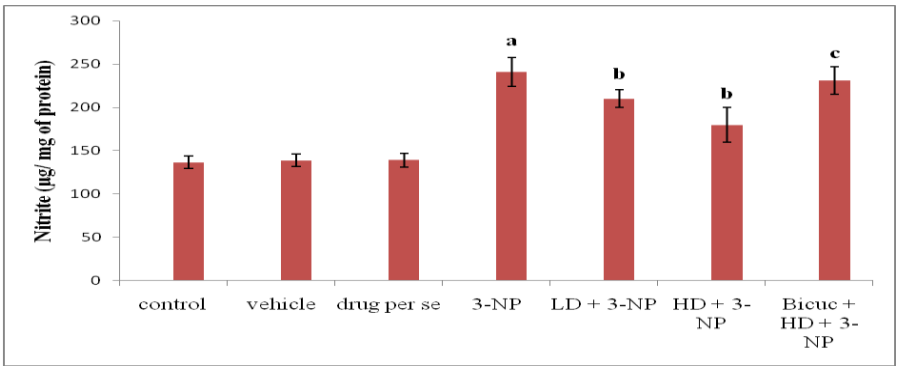

Figure 7: Effects of ascorbic acid on brain nitrite/ nitrate levels

Values are the mean ± SEM; a= p< 0.05 as compared to the normal control; b= p< 0.05 as compared to 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); F= 15.603; DF= 6, 35.

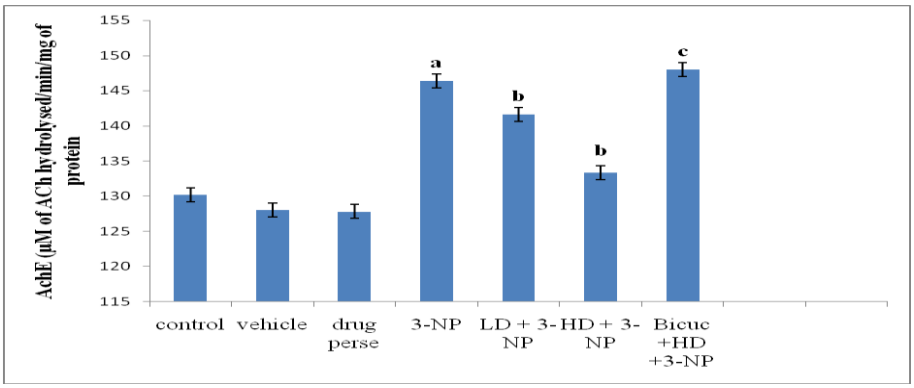

Figure 8: Effects of ascorbic acid on brain acetylcholinesterase (AchE) activity

Values are the mean ± SEM; a= p< 0.05 as compared to the normal control; b= p< 0.05 as compared to 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide (1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); F= 7.383; DF= 6, 28.

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

3-NP (10 mg/kg, *i.p*) administration caused significant (P<0.05) increase in acetylcholinesterase enzyme levels of test animals when compared to control group. Ascorbic acid treatment significantly (P<0.05) attenuated acetylcholinesterase enzyme activity as compared to 3-NP treated rats. Moreover, there was no significant effect of ascorbic acid (200 mg/kg, *i.p*) *per se* and vehicle treatment on brain acetylcholinesterase levels as compared to control group. Furthermore, treatment with bicuculline (1 mg/kg, *i.p*) abolished the ascorbic acid mediated reduction in acetylcholinestrase enzyme levels (Figure 8).

Systemic administration of 3-NP (10 mg/kg, i.p) caused a marked increase in MPO activity as compared to the control group. Further, there were no alterations in the brain MPO activity due to ascorbic acid (200 mg/kg, *i.p*) *per se* and vehicle treatment as compared to control rats. Ascorbic acid (100 mg/kg and 200 mg/kg, *i.p*) administration for 14 days, however, significantly (P<0.05) prevented the increase in MPO activity, with marked effect observed at the highest dose when compared to 3-NP treated group. Moreover, treatment with bicuculline (1 mg/kg, *i.p*) abolished the ascorbic acid mediated reduction in brain MPO activity (Figure 9).

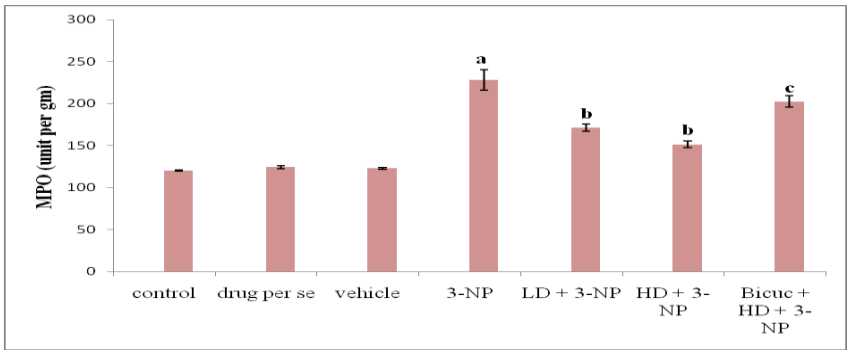

Figure 9: Effects of ascorbic acid on myeloperoxidase activity

Values are the mean ± SEM; a= p< 0.05 as compared to the normal control; b= p< 0.05 as compared to 3-NP injected group; c= p< 0.05 as compared to HD+ 3-NP group; Vehicle = DMSO- dimethyl sulphoxide(1% v/v, *i.p*); 3-NP = 3-nitropropionic acid (10 mg/kg, *i.p*); AA *per se*= ascorbic acid (200 mg/kg, *i.p*); LD = low dose ascorbic acid (100 mg/kg, *i.p*); HD = high dose ascorbic acid (200 mg/kg, *i.p*); Bicuc = bicuculline (1 mg/kg, *i.p*); F= 1129; DF= 6, 35

.

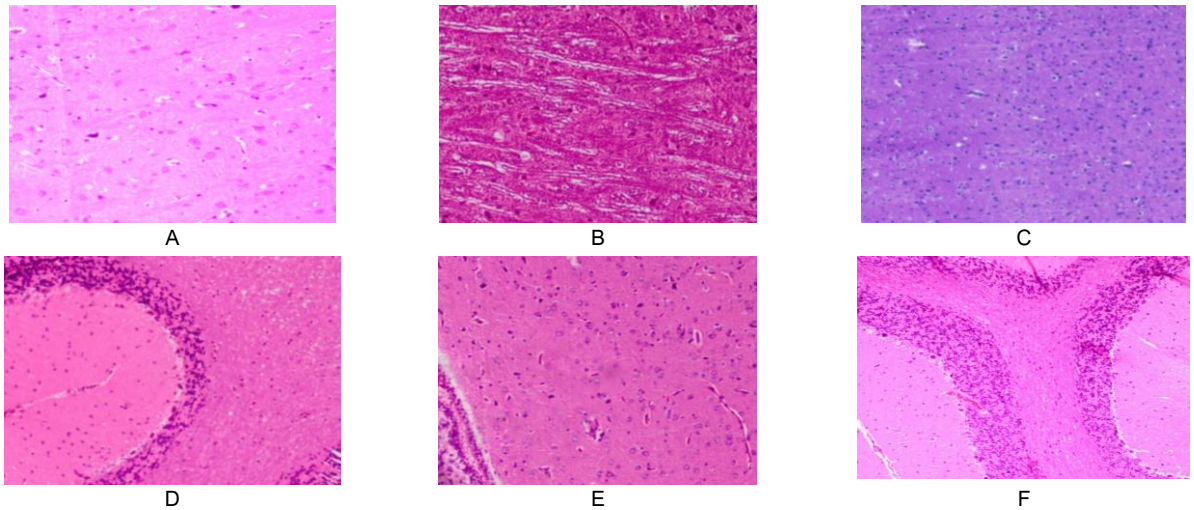

Figure 10: Effects of pharmacological intervention on brain histopathology (Haematoxylin and Eosin Staining)

A = untreated control brain; B = 3-NP (10 mg/kg/day, i.p, 14 days) treated brain; C= drug per se (200 mg/kg/day, i.p, 14 days) treated brain; D= 3-NP (10 mg/kg/day, i.p, 14 days) + ascorbic Acid (100 mg/kg/day, i.p, 14 days) treated brain; E = 3-NP (10 mg/kg/day, i.p, 14 days) + ascorbic acid (200 mg/kg/day, i.p, 14 days) treated brain; F = bicuculline (10 mg/kg/day, i.p, 14 days) and ascorbic acid (200 mg/kg/day, i.p, 14days) and 3-NP (10 mg/kg/day, i.p, 14 days) treated brain

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

These findings justified the use of 3-NP to develop animal model which impersonate the prominent features of HD. Moreover, other biochemical parameters such asacetylcholinesterase (AChE) and myeloperoxidase (MPO) from present study with stand the claim that neurodegeneration has been achieved in experimental animals (Figure 10). Administration of 3-NP resulted in increased AChE levels and increased MPO activity in study animals. There was no change in the initial and final body weight of the vehicle treated animals. However, 3-NP treatment caused a significant decrease in body weight on day 15th as compared to vehicle treated group.

There is considerable evidence that oxidative damage significantly contributes to the pathogenesis of several neurodegenerative diseases including HD. Remarkable results have been attained in the present investigation using ascorbic acid. Neurodegenerative diseases which exhibit high oxidative stress constantly consume ascorbic acid available in the brain [17]. When ascorbic acid is used as an antioxidant, it is oxidized. Therefore in the presence of high levels of ROS no ascorbic acid remains available to modulate neuronal metabolism. Thus oxidative stress, elevated ROS production and the failure of homeostatic systems for ascorbic acid recycling are crucial aspects in the progression of neurodegeneration. Ascorbic acid an antioxidant has shown protective effects against 3-NP induced neurodegeneration as well as loss of cognition and motor activity in rats. However, pretreatment with bicuculline a selective $GABA_A$ receptor antagonist abolished the ascorbic acid mediated protection that is signified by ↓GSH, ↑AchE, ↑MDA, ↑MPO and ↑nitrite levels when compared to high dose treated animals. The results of the present study demonstrate that ascorbic acid provides neuroprotection against 3-NP induced HD in rats and this protection is mediated via activation of $GABA_A$ receptors.

Conclusion

In conclusion, the results of the present study demonstrate that ascorbic acid has shown neuroprotective effects against 3-NP induced behavioural and biochemical alterations similar to huntington's disease in rats and the said role of ascorbic acid involves the activation of $GABA_A$ receptors. Taking into consideration the role of ascorbic acid here described, as an antioxidant molecule, a neuromodulator of synaptic activity and its function on the metabolic switch in neurons during brain activity and resting conditions, this molecule appears as a key factor in physiological and pathological conditions. Therefore, it is important to continue the efforts to thoroughly evaluate the roles of ascorbic acid for the development of new therapies against neurodegenerative disorders.

References

1. Yohrling GJ, Jiang GC, DeJohn MM, Miller DW, Young AB, Vrana KE, Cha JH. Analysis of cellular, transgenic and human models of huntington's disease reveals tyrosine hydroxylase alterations and substantia nigra neuropathy, Brain Res Mol Brain Res 2003;1119: 28-36
2. 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: 4181-4192.
3. Bossi SR, Simpson JR, Isacson O. Age dependence of striatal neuronal death caused by mitochondrial dysfunction, Neuroreport 1993; 4: 73-76.
4. Pinto AC, Acuna AI, Beltran FA, -Diaz LT, Castro MA. Old things new view: Ascorbic acid protects the brain in neurodegenerative disorders. Int J Mol Sci 2015; 16: 28194–28217.
5. Kumar P, Kumar A. Neuroprotective effect of cyclosporine and FK506 againt 3 nitropropionic acid induced cognitive dysfunction and glutathione redox in rat: possible role of nitric oxide. Neurosci Res 2009; 63: 302-314.
6. Kumar A, Dogra S. Neuroprotective effect of Carvedilol, an adrenergic antagonist against colchicine induced cognitive impairment and oxidative damage in rat. Pharmac Biochem Behav 2009; 92: 25-31.
7. SK Kulkarni. Hand Book of Experimental Pharmacology, 3rd edition, Vallabh Prakashan, 1999, 131-134
8. Reddy DS, Kulkarni SK. Possible role nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging- and dizocilpineinduced learning impairment. Brain Res 1998; 799: 215-229.
9. Nichans WG, Samuelson BJ. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 6:126-130.
10. Beutler E, Duran O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein Measurement with the Folin Phenol Reagent. J Biol Chem 1951; 193: 265-275.
12. Green LC, Wagner DA, Godowsky J, Skipper PL, Wishnok JS, Tannenbaum, SR. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal Biochem 1982; 126:131-138.
13. Ellman GL, Courtney KD, Andres VJ, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88–90.
14. Koladiya RU, Jaggi AS, Singh N, Sharma BK. Beneficial effects of donepezil on vascular endothelial dysfunction-associated dementia induced by Lmethionine in rats. J

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com

Health Sci 2009; 55: 215-225.
15. Krawisz JE, Sharon P, Stenson WF. Qualitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 1984; 87:1344-1350.
16. Clayden EC. Practical section cutting and staining, 5th Edition, Churchill, livingstone, Edinburgh and London 1971: 270.
17. Covarrubias-Pinto A, Moll P, Solis-Maldonado M, Acuna AI, Riveros A, Paz MM, Papic E, Beltrán FA, Cepeda, C, Concha II, et al. Beyond the redox imbalance: Oxidative stress contributes to an impaired GLUT3 modulation in huntington's disease. Free Radic Biol Med 2015; 89: 1085-1096.

Acknowledgement

The author would like to pay their sincere thanks to colleagues and friends for their constant moral support and scientific advices during the whole work.

Conflict of Interest: None declared

Received: 07 December 2017, Revised: 20 December 2017, Accepted: 29 December 2017

Dharmender Jaglan, E-mail:- dharmenderjaglan@gmail.com