EVALUATING NEUROPROTECTIVE EFFECTS OF NEBIVOLOL AGAINST 3-NITROPROPIONIC ACID INDUCED HUNTINGTON'S DISEASE IN RATS

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ABSTRACT:

Neurodegeneration is defined as the progressive loss of structure and function of neurons including cell death. Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the unstable expansion of cytosine–adenine–guanine (CAG) repeats in the gene encoding for a protein called huntingtin (htt). The mutant protein might be responsible for alterations in some components of the cytoskeleton, thus leading to affected axonal transport and cell death. In order to study huntington's disease impaired energy metabolism model using 3-Nitropropionic acid (10 mg/kg, *i.p*., for 14 days) was considered. Nebivolol , a beta blocker, appeared to function as an antioxidant , antiproliperative and an agonist for ppar-γ was used to treat the symptoms of huntington's disease with the dose of 1 and 0.5 mg/kg, *p.o*.. BADGE (30 mg/kg, *p.o*.) was used as an antagonist acting on ppar-γ receptors.

Keywords: Antioxidant, BADGE, Nebivolol

INTRODUCTION:

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder, characterized by an array of different psychiatric manifestations, cognitive decline and choreiform movements (Harper *et al*., 2000). Two forms of HD, juvenile HD and adult HD, have been arbitrarily divided by the age of disease onset. The term 'juvenile HD' is generally applied to cases of HD with onset before 20 years of age. Presentation of juvenile HD commonly shows symptoms of mental disturbance and rigidity rather than choric movements, while HD presenting in mid life more frequently shows a relatively pure movement disorder, usually chorea (Kumar *et al*., 2009). The psychiatric problems include a variety of conditions that range from antisocial personality, psychosomatic disorder, delusional disorder, and affective disorder to schizophrenia. Although chorea is a cardinal sign of HD, other motor abnormalities such as rigidity, bradykinesia, dystonia, cerebellar ataxia and myoclonus are common in juvenile HD and occasionally present in the late stage of adult HD (Ribai *et al*., 2007). Family studies of HD in the pre-genetic era had documented that most juvenile and early-onset cases of HD were paternally transmitted and that there appeared to be anticipation, i.e. progressively earlier onset in successive generations. HD is a relentlessly progressive disease, and survival ranges between 10 and 17 years from the age at onset (Quarrell *et al*., 2009).

Biochemical alterations found in the caudate of patients with HD are a consequence of selective cell death in neurons from basal ganglia. These changes include decreased levels of γ-aminobutyric acid (GABA), its synthesis enzyme

glutamate decarboxylase (GAD), acetylcholine (ACh) and its synthesis enzyme choline-acetyl transferase (CAT), and some peptides specifically localized in middle-sized spiny neurons (Borovecki *et al*., 2005). In addition, important alterations in the number of N-methyl-D-aspartate receptors (NMDA) have been described, suggesting that some components of the glutamatergic transmission may be involved as causative factors in HD (Ellerby 2002).

Huntington's disease(HD) is an autosomal dominant neurodegenerative disorder cause by the unstable expansion of CAG repeat in the gene encoding a polyglutamine (poly Q) tract in a protein called huntingtin (Walling *et al*.,1998), resulting in neuronal dysfunction and death predominantly in striatum and cortex (DiFiglia et al., 1997). The HD gene (also known as IT-15) is located on the short arm of chromosome 4 (HDCRG, 1993). The CAG repeat region shows a range of 11-35 repeats in normal individuals, while a repeat number of greater than 35 indicate a very high probability of developing HD (Roze *et al*., 2008). The expanded CAG repeat tend to be unstable and show both somatic and germline instability, frequently expanding rather than contracting in successive transmissions through the generations of a family. This is called intergenerational mutation instability (Leeflang *et al*., 1995). Anticipation results from intergenerational mutation instability and its corresponding impact on phenotype (Pavese et al., 2006). A significant inverse correlation was found between age at onset of symptoms and CAG repeat number, a trend that is even stronger within the juvenile group (Lobsiger *et al*., 2007). It was also observed that the severity and progression of disease is correlated to the length of CAG repeats (Micallef and Blin, 2001).

3-Nitropropionic acid (3-NP), a mycotoxin which causes irreversible inhibition of succinate dehydrogenase, (Okun *et al*., 2004) may induce characteristic basal ganglion lesions in humans and animals. Due to selective involvement of the striatal neurons, 3-NP-treated experimental animals may present pathological features mimicking those of Huntington's disease in humans (Paradisi *et al*., 2008). The 3-NP model can mimic and reproduce the hyperkinetic and hypokinetic symptoms of HD, depending on the time and dose administered, thus allowing the initial (or early) and late phases of HD to be evaluated. The administration of 3-NP (10 mg/kg intraperitonelly for more than four doses) induces the onset of similar symptoms to hypokinetic symptoms of HD, while administration in two individual doses display similar symptoms to hyperkinetic symptoms of HD (Borlongan *et al*., 1997b). Some motor alterations may constitute resemblances to HD, experimental models must also be able to reconstruct cognitive aspects of this disorder, such as those of memory and attention alterations. 3-NP is a toxin that irreversibly inhibits the enzyme succinate dehydrogenase (SDH; E.C. 1.3.99.1) (Alston *et al*., 1977), which in turn is present in the internal face of the mitochondrial membrane and is responsible for the oxidation of succinate to fumarate. Inhibition of this enzyme invariably leads to neuronal death in caudate and putamen nuclei, triggering severe dystonia in children (Ludolph *et al*., 1991). 3-NP also induces caspase-9 activation, which in turn requires the simultaneous presence of Apaf-1, cytochrome c and ATP. In regard to oxidative damage, it is known that the alteration of oxidative metabolism by 3-NP induces oxidative and nitrative stress due to excessive ROS/RNS production and/or depletion of antioxidant systems. Therefore, oxidative damage has been largely linked with neuronal loss in the 3-NP model. Therefore, oxidative damage has been largely linked with neuronal loss in the 3-NP model. Moreover, calcium ions (Ca^{2+}) play an important role in this process since its homeostasis is altered by the neurotoxin, which triggers cytosolic increases from internal storages that lead to NOS activation via Ca^{2+}/calmodulin, and the subsequent production of NO. Additionally, increased Ca^{2+} concentrations due to the opening of both voltage-gated membrane channels and

voltage-gated N-methyl D-aspartate (NMDA) receptor-channel complex trigger excitotoxicity and associated events, including the activation of proteases involved in cell death such as calpains (Túnez *et al*., 2004b; La Fontaine *et al*., 2000). Calpains in turn mediate the degradation of different proteins, including Htt. Interestingly, the activity of this protein is unaffected in areas where 3-NP does not cause cell death (Biglan and Shoulson, 2007; Galas *et al.*, 2004) 3-NP and neurotoxicity.

Nebivolol is a new third generation cardioselective lipophilic beta-blocker. It reduces peripheral vascular resistance and has a vasodilatory effect. It has a negative chronotropic effect and a slight negative inotropic effect with an efficient blockade of sympathetic system activity. One of important effects of this drug is the stimulation of formation and secretion of nitric monoxide (NO), the substance that is a key substance in prevention of the endothelial dysfunction that is crucial for the commencement of the atherogenic disorder (KneæeviÊ A.2008). Nebivolol also appears to function as an antioxidant and is able to modify markers of oxidative stress. In addition, there was a reduction of reactive oxygen species and superoxide anions in endothelial cells exposed in vitro to oxidative stress after incubation of the cells with plasma from patients who received nebivolol (Przedborski *et al*., 2007). Apart from the well-documented clinical cardiovascular and non-vascular applications of β-adrenoceptor antagonist, there is growing body of data showing that the activation of central β-adrenoceptors may also be involved in the development/progress of epileptic phenomena. The high density of β-adrenoceptors occurs in all the subfields of the hippocampus known for its low seizures and dominant role in the propagation of seizures (McNamara, 1994).

Studies suggest that peroxisome proliferator-activated receptor gamma (PPAR-γ) exerts beneficial effects in patients with mild-to-moderate dementia (Kaur *et al.*, 2009; Escribano *et al*., 2010). The nuclear receptor PPAR-γ is a ligand-activated transcription factor and its biological actions are to regulate glucose and lipid metabolism and suppress inflammatory gene expression (Landreth *et al.*, 2008). PPAR-γ agonists have been reported to effectively attenuate oxidative stress, inflammation and apoptosis in the central nervous system (CNS) (Kaundal and Sharma, 2010). Several studies have documented that PPAR-γ activation can also prevent or attenuate neurodegeneration (Napolitano *et al.*, 2011). PPAR-γ is expressed widely in the CNS where it has a prominent role in the regulation of neuroprotection (Fatehi-Hassanabad *et al*., 2011; Glatz *et al*., 2010). PPAR-γ agonists hasve also been found to have excellent antioxidant activity (Nicolakakis *et al*., 2008; Li *et al*., 2010). Studies have also reported that PPAR-γ agonists exhibit anti-inflammatory properties which are due to negative regulation of the expression of pro-inflammatory molecules such as interleukin-1b (IL-1b), IL-6 and TNF-α. (Halvorsen *et al*., 2010; Zhang *et al*., 2010). Further, PPAR-γ is being considered as a novel target to manage cognitive decline in AD patients (Landreth. 2007; Sodh *et al.*, 2011; Landreth *et al.*, 2008). PPAR-γ agonists significantly reduced spatial memory impairment induced by the amyloid

burden, Aβ aggregates, Aβ oligomers, astrocytic and microglia activation (Toledo and Inestrosa., 2010). Nebivolol acts as a ppar-γ agonist and BADGE acts as antagonist.

MATERIAL AND METHODS:

Animals:

Male wistar rats obtained from National Institute of Pharmaceutical Education and Research (NIPER), Mohali and maintained in the central animal house of the facility of Chandigarh College of Pharmacy, Landran, Mohali, Punjab (1201/a/08/CPCSEA) and weighing between 180 and 220g, were used. Rats were kept under standard environmental conditions of constant temperature (23 ±1ºC) and humidity level adjusted to 60%. The animals were kept under standard conditions of 12 hr light and dark cycle with food and water ad libitum in plastic cages with soft husk bedding. All the experiments were carried out between 09:00 and 17:00 hr in semi sound proof laboratory conditions.

Drugs:

All drugs and reagents were freshly prepared before use and suitable precautions were taken during handling of drug and reagents. All chemicals and biochemical reagents of analytical grade and highest purity were used. 3-nitropropionic acid (Sigma-Aldrich corporation, India) was diluted with saline (adjusted pH 7.4 with NaOH) and administered intraperitoneally (*i.p.*). Nebivolol (1.0 mg/kg, *p.o.)* was suspended in 0.25% of carboxy methyl cellulose in 0.9% saline solution and were freshly prepared prior to oral administration in a volume of 0.5ml per 100g of body weight.

Study design:

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 (Kumar and Kumar, 2008). 3-NP injection was freshly prepared in normal saline (0.5 ml of normal saline per 100 gm of animal weight) before administration. Only rats with positive behavioural effects were included in the study.

All animals were acclimatized for at least 2 hrs before testing, unless otherwise specified in all the experiments. Animals were divided in six groups and each group consisted of five animals as approved by IAEC for the pre emptive paradigms. To evaluate the effects nebivolol (0.5mg/kg and 1mg/kg, *p.o.*) treatment was initiated on day 1 and continued till day 14. BADGE (a ppar-γ antagonist) was also given in a different group of animals before nebivolol (1 mg/kg, *p.o.*) and 3-NP (10 mg/kg, *i.p.*) for 14 days.

In present study,

Group 1- the control group of animals received equivalent volume of 0.25% of carboxy methyl cellulose in 0.9% saline solution before behavioural assessment.

Group 2- the 3-NP control received 3-NP (10 mg/kg, *i.p.*) once daily for a period of 14 days.

Group 3- Drug control group received nebivolol (1mg/kg, *p.o.*) once daily for 14 days.

Group 4- received nebivolol (0.5 mg/kg, *p.o.*) once daily followed by 3-NP (10 mg/kg, *i.p.*) after 1 hour for a period of 14 days.

Group 5- received nebivolol (1mg/kg, *p.o.*) once daily followed by 3-NP (10 mg/kg, *i.p.*) after 1 hour for a period of 14 days.

Group 6- received BADGE (30mg/kg/day, p.o.) 1 hour before nebivolol (1mg/kg/day, *p.o.*) followed by 3-NP after 1 hour for 14 days.

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 analysis:

Elevated plus maze paradigm:

It consists 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 1 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 arms is recorded as the initial transfer latency. The animals were allowed to explore the maze for 30 s after recording the initial transfer latency and then returned to its home cage. If the animal did not enter an enclosed arm within 90 s, it was gently pushed in to the enclosed arm and the transfer latency was assigned as 90 s. retention of memory was assessed by similarly placing a rat on an open arm, and noting the retention latency on day 5 and 15 days after the initial transfer latency (ITL). These times were referred to as the first retention transfer latency and second retention transfer latency, respectively (Kulkarni *et al*., 1993).

Morris water maze:

- Spatial navigation task:

The acquisition and retention of the spatial navigation 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 ± 2º C) 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 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 a day for 4 days. 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):

On day 5, after the initial acquisition latency (IAL), a rat was randomly released at centre of the pool and tested for the retention of the response. The time taken to reach the hidden platform on day 2 and 14 following initiation of 3-NP treatment was recorded and termed as the first retention latency and second retention latency, respectively (Frautschy *et al*., 2001).

Assessment of gross locomotor activity using Actophotometer:

The locomotor activity was monitored using actophotometer on 1st, 5th and 15th day of 3-NP administered and nebivolol treated groups. 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 (Reddy *et al.*, 1988).

Collection of blood samples:

Animals were sacrificed by cervical dislocation and the brains were removed and homogenized in phosphate buffer (pH 7.4). The homogenates were then centrifuged at 3000 rpm for 15 mins. The supernatant of the homogenates were used for biochemical estimations acetylcholinesterase (AChE), brain total protein, thiobarbituric acid reactive species (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), nitrite and MPO) as per the methods described below.

Biochemical analysis:

Estimation of brain acetylcholinestrase (AChE) activity:

AChE is a marker of loss of cholinergic neurons in the forebrain. The whole brain AChE activity was measured by method of Ellman *et al*. (1961) with slight modifications (Koladiya *et al*., 2009; Sain *et al*., 2011). This was measured on the basis of the formation of yellow colour due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from sacetylthiocholine iodide in the presence of brain cholinesterase was measured using a spectrophotometer. 0.5 ml of supernatant of the brain homogenate was pipetted out into 25 ml volumetric flask and dilution was made with freshly prepared DTNB {5,5'-Dithiobis (2-nitrobenzoic acid)} solution. From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tube, 2 drops of serine solution were added. 1ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both of the test tubes and incubated for 10 min at 30º C. the test tube containing serine solution was used for zeroing the calorimeter and change in absorbance per min of the sample was readed spectrophotometrically at 420 nm (UV-1800 spectrophotometer, Shimadzu, Japan). AChE was calculated by the following formula:

$$R = \frac{\delta\, O.D. \times Volume\ of\ assay}{E \times mg\ of\ protein}$$

Where R = rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolysed/minute/mg protein.

δO.D. = change in absorbance/min.

E = Extinction coefficient = (13,600/M/cm)

Estimation of brain total protein:

The brain total protein content was determined by the method of lowry *et al*. (1951)

with slight modifications using bovine serum albumin (BSA) as a standard. 0.15 ml of supernatant of tissue homogenate was diluted to 1 ml with distilled water and then 5 ml of lowry reagent was added. The contents were mixed thoroughly. The mixture was allowed to stand for 15 mins at room temperature (37ºC). Then, 0.5 ml of 1:1 v/v

diluted Folin-ciocalteu's reagent was added. The content was vortexed vigorously and incubated at room temperature (37ºC) for 30 mins. The protein content was determined spectrophotometrically at 750 nm (UV-1800 spectrophotometer, Shimadzu, Japan) against suitably prepared blank. A standard curve using 0.2-2.4 mg/ml of BSA was plotted. The amount of total protein was expressed in mg/ml.

Estimation of thiobarbituric acid reactive species (TBARS) level:

The whole brain TBARS level, an index of lipid peroxidation was described by Niehius and Samuelson, (1968). In this method, 0.1 ml of brain homogenate supernatant was treated with 2 ml of (1:1:1) thiobarbituric acid – trichloroacetic acid (TBA-TCA-HCl) reagent. TBARS reagent was prepared by mixing equal volumes of TBA (0.37%), TCA (15%) and HCl (0.25 N). then, the mixture was placed in water bath for 15 mins, cooled and centrifuged at room temperature for 10 mins at 1000 rpm. Finally, the absorbance of clear supernatant was measured against refrence blank at 535 nm (UV-1800 spectrophotometer, Shimadzu, Japan). The absorbance from a standard curve generated using 1,1,3,3 tetra-methoxy propane (TMP) as standard (range = 1 nmol-10 nmol) was extrapolated. Results were expressed as nmol/mg of protein.

Estimation of myeloperoxidase activity:

The myeloperoxidase (MPO) activity which is measured as an index of neutrophil accumulation was measured using method of Krawisz *et al*., (1984). In the pellet obtained after tissue homogenization 10 mL of ice-cold potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB) and 10 mM ethylene diamine tetra acetic acid (EDTA) was added and subjected to one cycle of freezing and thawing and then sonication for 15 s was done. The contents were centrifuged at 15,000 g for 20 minutes. 0.1 mL of supernatant obtained after centrifugation was mixed with 2.9 mL of phosphate buffer containing 0.16 mg/mL of *o*-dianisidine hydrochloride and 0.0005% hydrogen peroxide (H_2O_2). The change in absorbance was measured using spectrophotometer at 460 nm. The MPO activity was expressed as unit per gram of tissue weight where 1 unit is the quantity of enzyme able to convert 1 μM of H_2O_2 to water in 1 minute at room temperature. The calculation of MPO activity was done using formula:

MPO activity (U/g) = X / Weight of the tissue

Where X = 10 x change in absorbance per minute/volume of supernatant taken in mL

Estimation of nitrate level:

The total nitrite concentration in brain exudate was measured by the method described by Green *et al*., 1982. The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined using a colorimetric assay with the Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% phosphoric acid). Equal volumes of supernatant and the Griess reagent were mixed. Then, the mixture was incubated for 10 min at room temperature in the dark, and the absorbance was measured at 540 nm using a spectrophotometer (UV-1700 spectrophotometer, Schimadza). The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as a percentage of control. An average result was recorded.

Estimation of brain reduced glutathione (GSH) level:

The whole brain GSH level was estimated by method of beutler *et al.* (1963). The supernatant of tissue homogenates were mixed with trichloroacetic acid (10% w/v) in the ratio of 1:1. Then, sample were centrifuged at 1000 × g for 10 min at 4º C. to 0.5 ml of this supernatant, 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.25 ml of freshly prepared 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) were added. The absorbance was measured spectrophotometrically at 412 nm (UV-1800 spectrophotometer, Shimadzu, Japan). Different concentrations of reduced glutathione (GSH) standard were also being processed similarly to prepare a standard curve (10-100 µm). Results were expressed as micromoles of reduced glutathione per mg of protein

Histopathological examination:

The second portion of each brain was fixed in formalin buffer (10%) for 24h. The brains were washed in tap water and then dehydrated using serial dilutions of alcohol (methyl, ethyl, and absolute ethyl). Specimens were cleared in xylene and embedded in paraffin in a hot air oven at 561C for 24h. Paraffin bees wax blocks were prepared for sectioning at 4mm using a microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin stains (Banchroft, *et al*., 1996) for histopathological examination using a light microscope.

Statistical evaluation:

In the present study, all results were expressed as mean± Standard error of the mean. Data were analyzed by one-way analysis of variance using the statistical package for social sciences (SPSS) program, version 11 (SPSS Inc., Chicago, Illinois, USA). The least significant difference was calculated to compare significances between the groups. The square root transformation (%) was calculated according to the method described by Jones *et al*. (2006). Thereafter, comparisons between more than two groups were made using analysis of variance, followed by Dunn's multiple comparison test or the Tukey–Kramer multiple comparison test to analyze the results of the T-maze test. A difference was considered significant at a P value of less than 0.05.

RESULTS:

Effect of nebivolol on body weight.

There was no change in the initial and final body weight of control animals. However, 3-NP treated rats have shown a significant ($p < 0.05$) decrease in body weight (% change in body weight) on 15th day as compared to control group. Nebivolol per se (1 mg/kg, *p.o.*) and vehicle (0.25% CMC) treatment had no effect on body weight. However, pretreatment with nebivolol (0.5 mg/kg, 1 mg/kg, *p.o.*) on 3-NP treated rats significantly ($p < 0.05$) and dose dependently prevented the decrease in body weight. Further, administration of BADGE (30 mg/kg, p.o.) abolished the effect of nebivolol.

Effect of nebivolol on memory and learning.

In this study, the mean 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 90 s. Following training, the control and nebivolol per se (1 mg/kg, *p.o.*) rats entered the closed arm quickly, and the mean retention transfer latencies (1st RTL and 2nd RTL) to enter the closed arm on days 2, 5 and 14 were shorter as compared to the ITL on day 1 of each group. In contrast, the 3-NP treated rats performed poorly throughout the experiment, and an increase in the mean RTL on days 2, 5 and 14 were noted as compared to the pre-training latency on day 1, demonstrating 3-NP induced cognitive dysfunction. Moreover, nebivolol pretreatment (0.5 mg/kg and 1 mg/kg, *p.o.*) significantly ($p < 0.05$) abolished 3-NP induced increase in mean RTL indicating an improvement in cognition. Further BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

In the morris water maze (MWM), the mean retention latencies to escape onto hidden platform were not altered in either of vehicle treated and nebivolol per se treatment rats on day 2, 5 and 14 respectively, when compared with IAL on day 1. However, 3-NP caused significant ($p \leq 0.05$) cognitive impairment as indicated by increased mean RTL when compared with control animals. Furthermore nebivolol pretreatment (0.5 mg/kg and 1 mg/kg, *p.o.*) improved retention of spatial navigation task when compared with 3-NP treated group. Further BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

Effect of nebivolol on gross locomotor activity.

3-NP (10 mg/kg, *i.p.*) administration repeatedly for 14 days significantly ($p < 0.05$) decreased the total activity counts compared to control group. Pretreatment with nebivolol (0.5 mg/kg and 1 mg/kg, *p.o.*) significantly ($p \leq 0.05$) attenuated the 3-NP-induced decrease in motor activity in a dose dependent manner on day 2 , 5 and 14. Whereas, nebivolol per se (1 mg/kg, *p.o.*) and vehicle (0.25% CMC) had no effect on gross behavioural activity when compared with control group. Further, BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

Effect of nebivolol on brain lipid peroxidation.

Systemic administration of 3-NP caused a marked increase in free radical generation and lipid peroxidation in addition to a decline in antioxidant defense, as indicated by a significant ($p < 0.05$) rise in brain MDA levels when compared to control group rats. Futher, there were no alterations in the brain MDA levels due to vehicle and nebivolol (1 mg/kg) per se treatment. Nebivolol (1 mg/kg, *p.o.*) administration for 14 days, however, significantly ($p < 0.05$) prevented the increase in MDA levels, with the marked effect observed at the highest dose used when compared to 3-NP treated group. Moreover, BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

Effect of nebivolol on brain reduced glutathione (GSH)

GSH levels in 3-NP administered rats have shown significant ($p < 0.05$) decrease as compared with control group. Whereas there was no significant effect of nebivolol (1 mg/kg, *p.o.*) per se and vehicle treatment on brain GSH levels. Pretreatment with nebivolol (0.5 mg/kg and 1 mg/kg, *p.o.*) significantly ($p < 0.05$) attenuated 3-NP mediated

decrease in brain GSH levels in a dose dependant manner. Moreover, BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

Effect of nebivolol on brain nitrite levels.

Systemic 3-NP (10 mg/kg, *i.p.*) administration caused a significant ($p < 0.05$) increase in brain nitrite levels. However there was no significant effect of nebivolol (1 mg/kg, *p.o.*) per se treatment on brain nitrite levels as compared to vehicle-treated rats. Pretreatment with nebivolol (0.5 mg/kg and 1 mg/kg, *p.o.*) significantly ($p < 0.05$) attenuated the 3-NP induced increase in nitrite levels. Moreover, BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

Effect of nebivolol on brain acetylcholinesterase (AChE) level .

AChE levels in 3-NP (10 mg/kg, *i.p.*) administered rats have shown significant (significant ($p < 0.05$) increase as compared to control group. Whereas, there was no significant effect of nebivolol (1 mg/kg, *p.o.*) per se and vehicle treatment on brain AChE levels. Pretreatment with nebivolol (0.5 mg/kg and 1 mg/kg, *p.o.*) significantly attenuated 3-NP mediated increase in brain AChE levels in a dose dependent manner. Moreover, BADGE (30 mg/kg, *p.o.*) administration ameliorated the protective effects of nebivolol.

Effect of nebivolol on brain Myeloperoxidase (MPO) activity

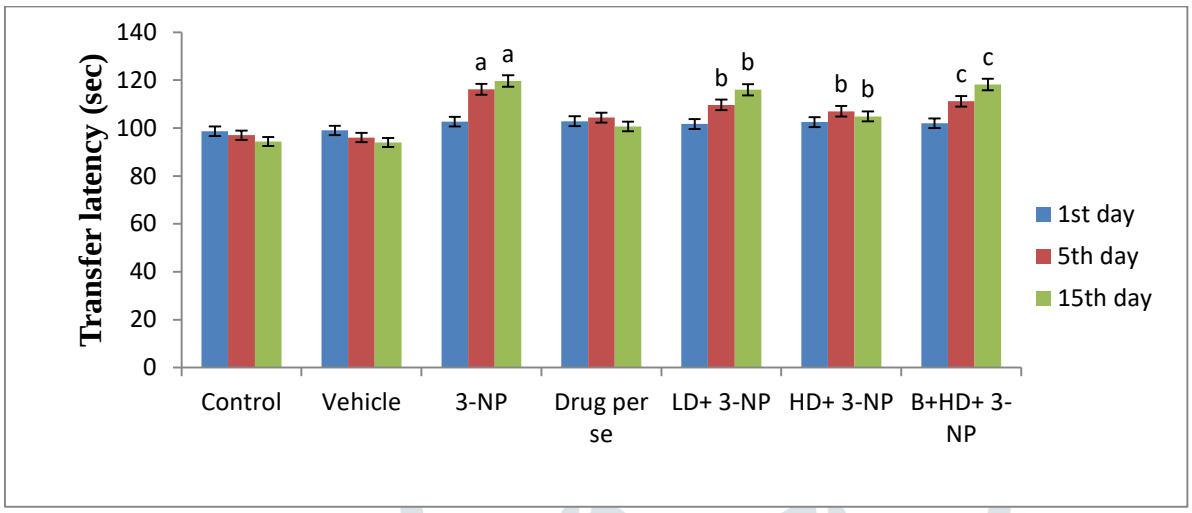
Systemic administration of 3-NP (10 mg/kg, *i.p.*) caused a marked increase in MPO activity as compared to the control group. Further, there were no alterations in the brain MPO activity due to nebivolol (1 mg/kg, *p.o.*) *per se* and vehicle treatment as compared to control rats. Nebivolol (1 mg/kg and 0.5 mg/kg, *p.o.*) 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 BADGE (30 mg/kg, *p.o.*) abolished the ascorbic acid mediated reduction in brain MPO activity.

GRAPHS AND TABLES:

Groups	Treatment	Initial Weight (gms)	Final Weight (gms)
I	Control	222.5 ± 6.5	219 ± 7.4
II	Vehicle	220.6 ± 9.2	218 ± 8.3
III	3-NP	221.6 ± 8.8	200 ± 8.4 [a]
IV	Drug *per se*	220 ± 10.4	222 ± 8.1
V	LD+3-NP	220.6 ± 9.7	218.3 ± 6.5 [b]
VI	HD+3-NP	220.0 ± 7.7	217 ± 7.6 [b]
VII	B.A.D.G.E+ HD+3-NP	221.6 ± 8.5	210 ± 6 [b]

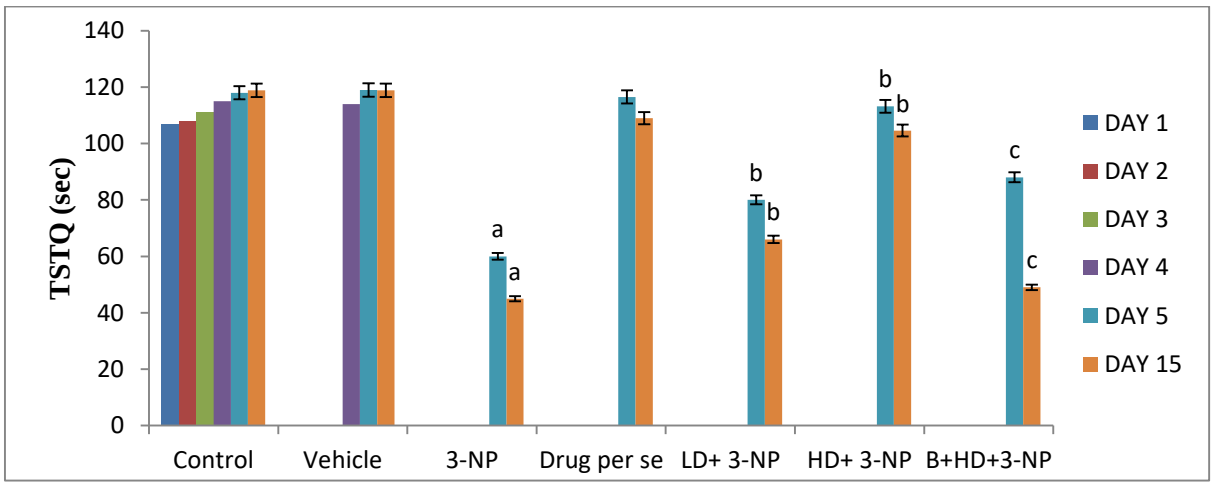
Effect of various interventions on body weight

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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.



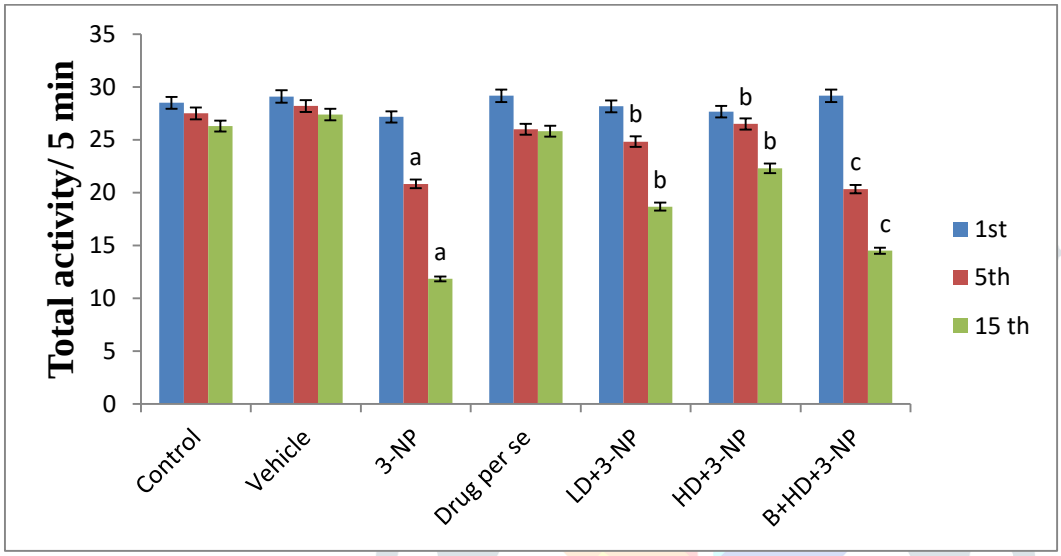
Effect of nebivolol on memory using elevated plus maze in rats.

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; 1st day (F= 1.229 ; DF= 6, 35); 5th day (F= 8.904 ; DF= 6, 35); 15th day (F= 37.648 ; DF= 6, 35).



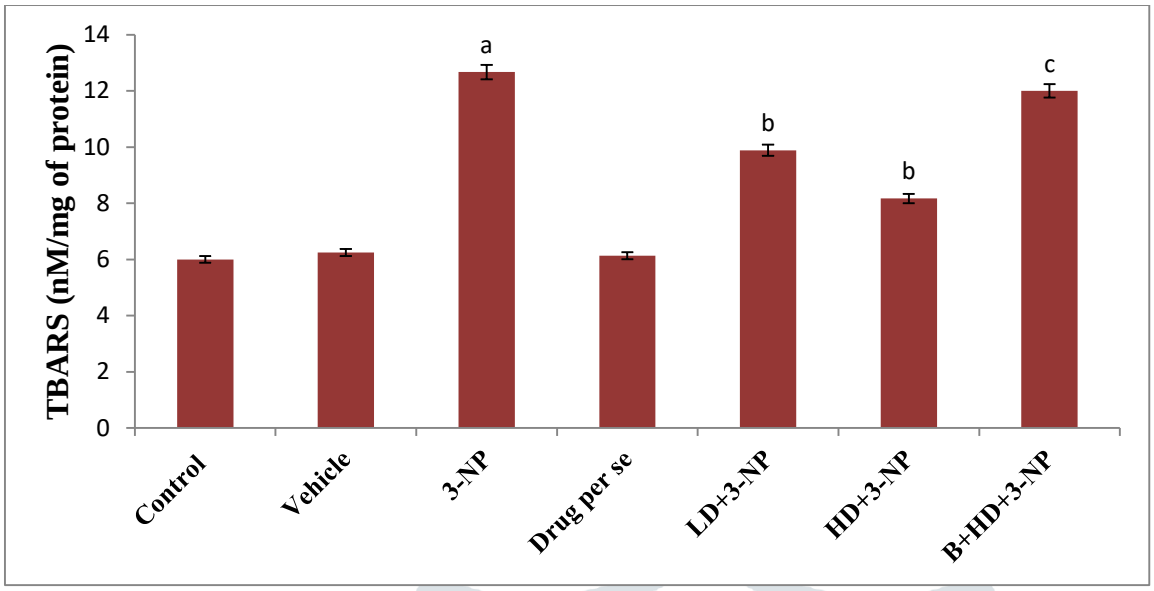
Effect of nebivolol on learning and memory using morris water maze in rats.

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; 5th day (F= 8.78 , DF= 6, 35); 15th day (F= 8.67 , DF= 6, 35).



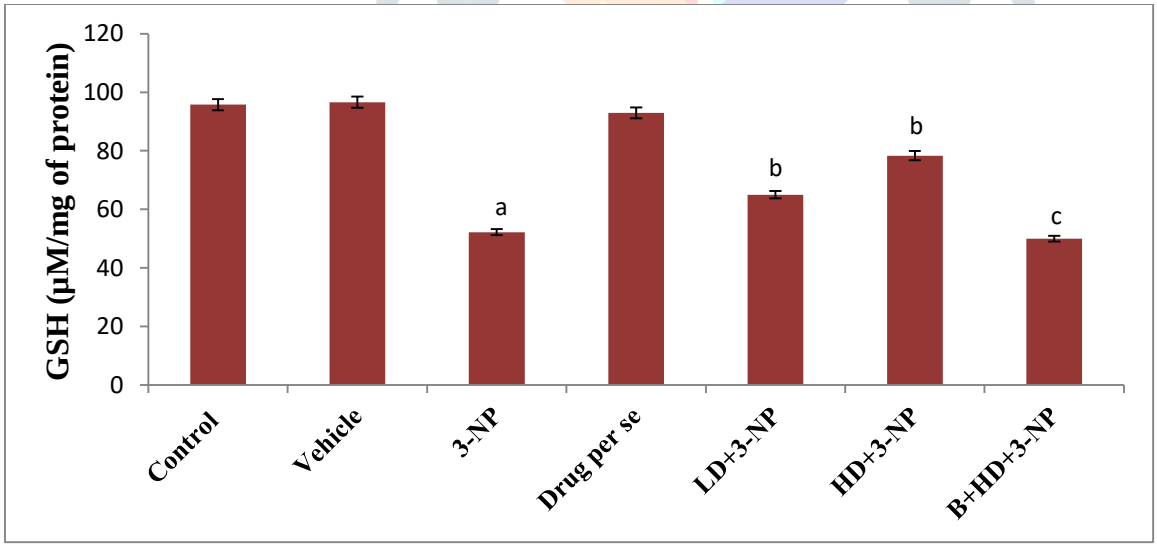
Effect of nebivolol on gross locomotor activity using actophotometer in rats.

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; 1st day (F= 0.398 ; DF= 6, 35); 5th day (F= 1.047 ; DF= 6, 35); 15th day, (F= 14.184 ; DF= 6, 35).



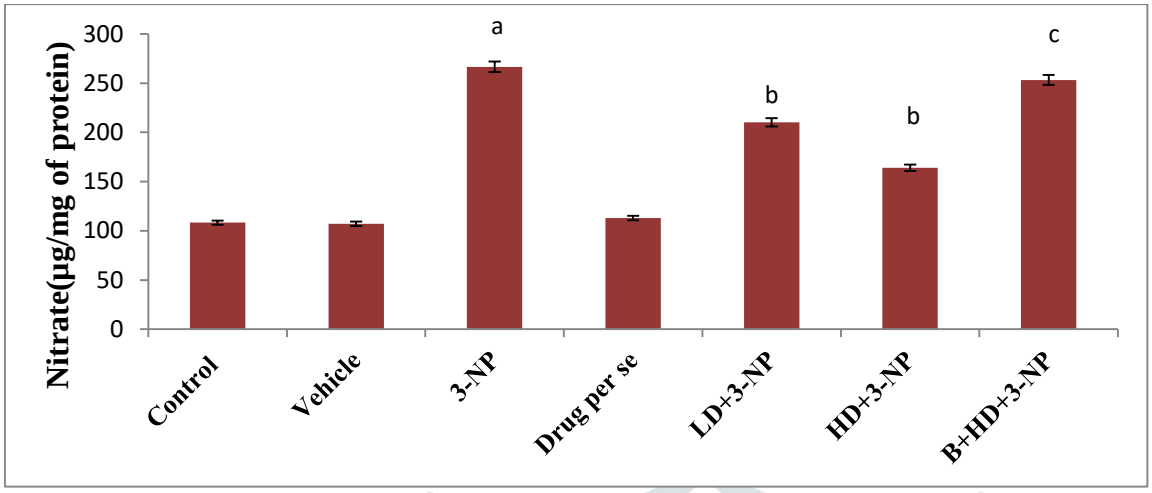
Effect of Nebivolol on lipid peroxidation (TBARS) level in rats.

Values are the mean ± SEM**;** Vehicle = 0.25% CMC in 0.9% saline *p.o.***;**3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; F= 227.23; DF= 6, 35.



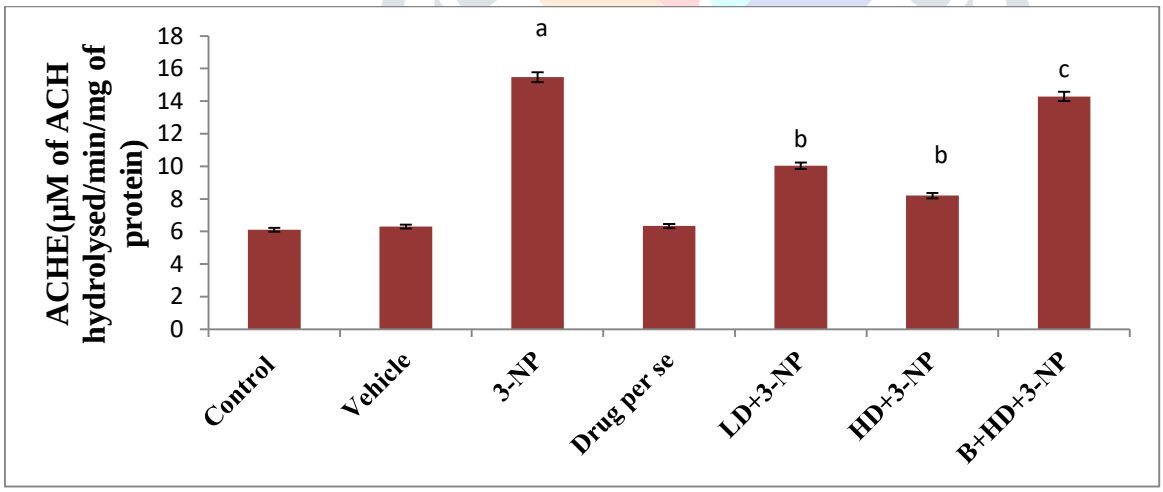
Effect of Nebivolol on glutathione (GSH) level in rats.

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; F= 47.773; DF= 6, 35.



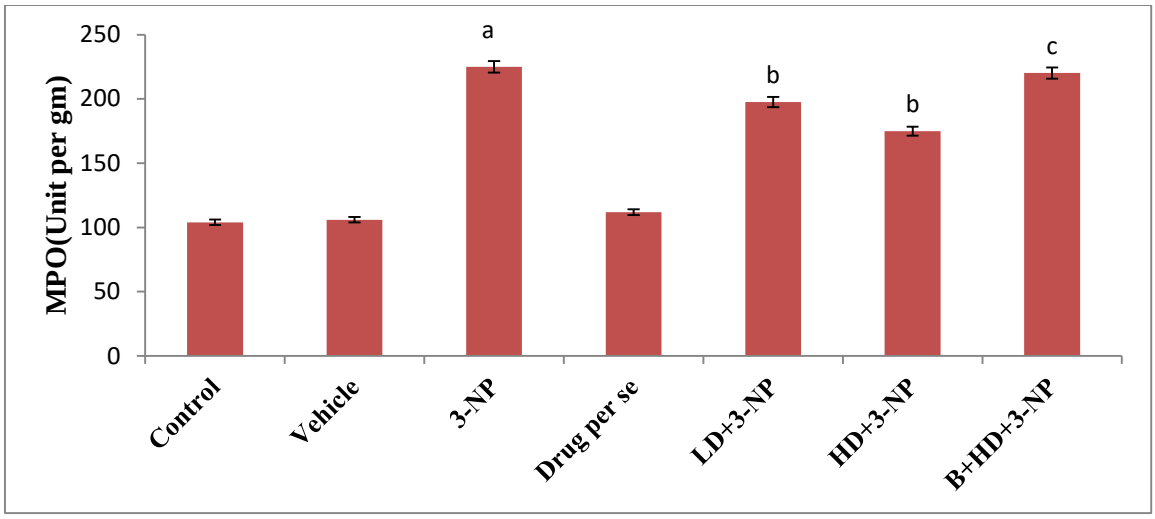
Effect of Nebivolol on nitrate/nitrite levels in rats.

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; F= 48.217; DF= 6, 35.



Effect of Nebivolol on acetylcholinesterase (ACHE) in rats.

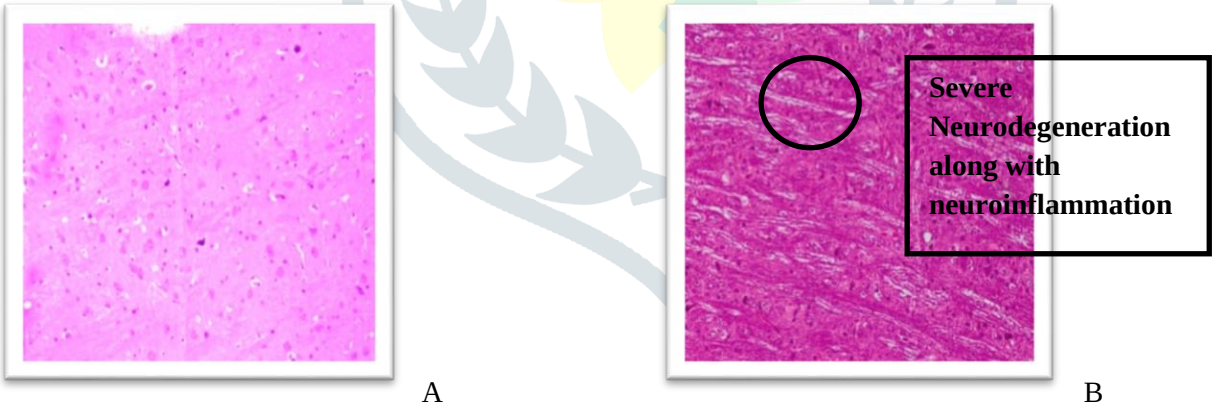
Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; F= 100.86; DF= 6, 35.



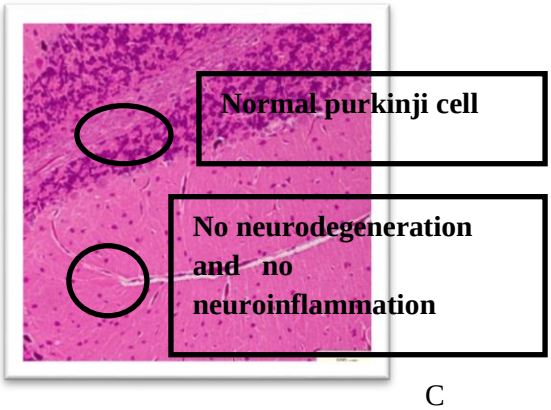
Effect of Nebivolol on myeloperoxidase MPO levels in rats.

Values are the mean ± SEM; Vehicle = 0.25% CMC in 0.9% saline *p.o.*; 3-NP = 3-Nitro Propionic acid (10 mg/kg, *i.p.*); Drug *per se*= Nebivolol (1 mg/kg, *p.o.*); LD = Low dose Nebivolol (0.5 mg/kg, *p.o.*); HD = High dose Nebivolol (1 mg/kg, *p.o.*); B = BADGE = Bisphenol-A-diglycidyl ether (30 mg/kg, *p.o.*); n = 6; 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; F= 176.74; DF= 6, 35.

Histopathological analysis:



A B



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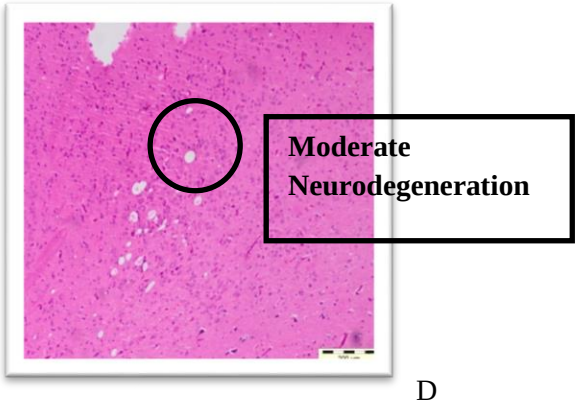

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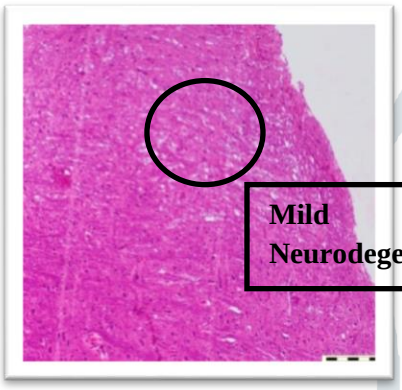

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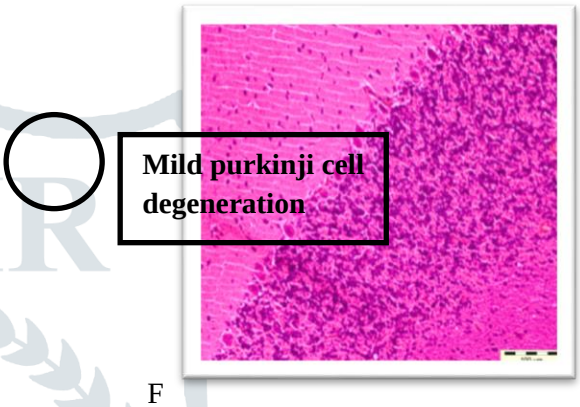
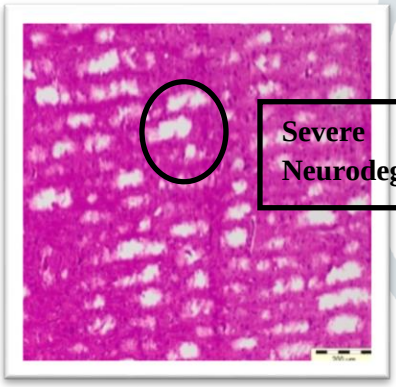


F



G

Slide 1: control (Fig. A)

The section of cerebrum region shows no degeneration. No meningitis is seen. Very less inflammatory cells are seen *i.e* no neuro-inflammation. Cerebellum have shown normal purkinji cells.

Slide 2: 3-NP (Fig. B)

The animals were injected with 3-nitropropionic acid 10 mg/kg daily for 14 days the brain cerebrum showed severe meningeal congestion, meningitis along with haemorrhage. Also there is infilteration of lympho-mononuclear cells in the cerebral parenchyma and vascular degeneration is seen. Cerebellum showed purkinji cell degeneration.

Slide 3: Drug per se (NBV) (Fig C)

The animals were treated with nebivolol 1mg/kg daily for 14 days. There was no degeneration in cerebrum and cerebellum. Purkinji cells were normal in cerebellum and also inflammatory cells were not seen.

Slide 4: 3-NP + low dose NBV (Fig. D)

The animals were given nebivolol 0.5 mg/kg along with 3-NP 10 mg/kg daily for 14 days. There was moderate cerebral degeneration. Cerebellum showed mild purkinji cell degeneration. Mild brain meningeal congestion and haemorrhage was also seen.

Slide 4: 3-NP + high dose NBV (Fig. E, F)

The animals were given nebivolol 1mg/kg along with 3-NP 10 mg/kg daily for 14 days. There was negligible cerebral degeneration. Cerebellum showed normal purkinji cells and very mild degeneration. Mild brain meningeal congestion and haemorrhage seen.

Slide 5 : B.A.D.G.E + 3-NP+ NBV (Fig. G)

The animals were given bisphenol A diglycidyl ether(B.A.D.G.E., an antagonist for ppar-γ), nebivolol (1mg/kg, p.o) and 3-NP(10 mg/kg, i.p) daily for 14 days. The cerebral section showed severe haemorrhage along with severe degeneration. Cerebellum also showed purkinji cell degeneration and meningitis was observed indicating that BADGE prevented action of nebivolol and that nebivolol was acting through ppar-γ receptors.

DISCUSSION:

The animal model used in present study employs 3-nitropropionic acid (3-NP) to induce huntington's disease (HD). 3-NP is a well-known neurotoxin which produces behavioural and biochemical symptoms similar to HD in animals and humans (Patocka et al., 2000). 3-NP promotes complex-II inhibition and causes increased sensibility of NMDA-R (excitotoxicity) (Deshpande, 2006). 3-NP produces selective lesions in basal ganglia (striatum), cortex and hippocampus (Beal. 1992; Colle *et al.*, 2013). It has been reported that striatal lesions and bradycardia is responsible for reducing rat appetite and food intake (Pubill *et al*., 2001). Patients with later stage of HD exibit dysphagia and loss of body weight (Saydoff *et al*., 2006).

3-NP toxicity affects microglia, astrocytes and neurons, and causes secondary excitotoxicity by making neurons more vulnerable to endogenous basal levels of glutamate, while prompting a reduction of ATP availability (Deshpande, 2006). Microglia activation is accompanied by increased ROS production, thereby allowing these glial cells to participate in the 3-NP-induced neurotoxicity and neurodegeneration (Ryu *et al*., 2003; Bantubungi *et al*., 2005; Nishino *et al*., 2000). The neurotoxin induces cell death through necrosis and apoptosis, a couple of processes also observed in the course and evolution of HD (Pang *et al*., 1997).

Elevated plus maze (EPM) and morris water maze (MWM) are most widely used models to test learning and memory. In EPM, the mean initial transfer latency (ITL) on day 1 before 3-NP treatment for each rat was relatively stable and showed no significant variation. The 3-NP administration group showed significant increase in value on day 10th and 15th as compared to vehicle treated rats, indicating impairment in learning and memory. In MWM test, mean transfer latency on the 1st and 4th day of training was same in all the groups. However, memory retention and time spent in target quadrant was significantly decreased on 5th, 10th and 15th day in 3-NP treated animals as compared to control animals.

3-NP (10 mg/kg, i.p.) administration for consecutive 14 days significantly impaired the memory on 15th day as observed in the MWM and EPM paradigms. As illustrated by increase in ELT of days in water maze along with increased latency in EPM when compared to control animals and our study withstand earlier reports stating that 3-NP produces lesions in hippocampal CA1 and CA3 pyramidal neurons, the area of the brain that is associated with cognitive performance (Kumar *et al*., 2007). It is also well known that striatal lessions induced by systemic administration of 3-NP in rats leads to decrease in the locomotor activity in rats (Kumar *et al*., 2007). This could also be simply because of decreased energy metabolism after 3-NP treatment. Our studies have shown that 3-NP treatment resulted in significant reduction in locomotor activity and grip strength as accessed by using actophotometer and rota-rod apparatus respectively. Typically, course of HD also begins with involuntary movements (chorea) that eventually progresses to rigidity and dystonia closely modelled by 3-NP administration. 3-NP showed significant increase in MPO which signifies inflammation, increase MDA level, elevated nitrate and also elevated AChE levels. 3-NP decreased body weight and also decreased GSH levels which further shows that it leads to oxidative stress.

Pretreatment with Nebivolol (NBV) significantly decreased the RTL of 15th day as compared to control group, indicating improvement in both learning and memory. It also significantly decreased the acquisition value on day 15th as compared to control group, indicating improvement in both learning and memory. NBV adminstration showed increase in GSH levels and also prevented weight loss. It significantly attenuated 3-NP induced increase in MPO levels, increased MDA levels, nitrate and AChE levels which results in allevation of oxidative stress, inflammation, and a part induction in neurodegeneration. NBV pretreament also improved the locomotor activity and grip strength in animals.

There is substantial evidence that oxidative damage significantly contributes to the pathogenesis of several neurodegenerative diseases including HD (Kumar *et al*., 2007; Kumar and Kumar, 2009). Excessive release of nitric oxide becomes toxic to striatal astrocytes, neurons and produces lesions. HD (Huntington's disease) is a progressive, debilitating, and fatal neurological disorder. HD is inherited in an autosomal dominant fashion. The mutated gene is located on the short arm of chromosome 4 and contains an expansion in the normal number of CAG (glutamine) repeats, generally 40 (The Huntington's Disease Collaborative Research Group, 1993). The protein encoded by the HD gene, htt (huntingtin), is normally a cytoplasmic protein closely associated with vesicle membranes and microtubules. Another pathological landmark of HD is the presence of aggregated forms of mutant htt in neurons. These aggregates comprise intranuclear [NII (neuronal intranuclear inclusion)] and cytoplasmic inclusions, as well as microaggregates (Rosas *et al*., 2005). Biochemical alterations found in the caudate of patients with HD are a consequence of selective cell death in neurons from basal ganglia which have a high content of these aggregates

(Rosas *et al*., 2005). There is severe mitochondrial damage in HD. Impaired energy metabolism can produce an oxidative stress, formation of ROS and RNS (Schulz *et al*., 1995; Broulliet *et al*., 2005) indicated by marked increase in MDA (a marker of lipid peroxidation) nitrite levels and depletion of GSH, which is suspected to be critically involved in neuronal cell death and indication of neurodegenerative diseases.

The peroxisome proliferator-activated receptor gamma (PPAR-γ) is a transcriptional factor that plays a key role in regulating genes involved in energy metabolism; recent studies demonstrated that PPAR-γ activation prevented mitochondrial depolarization in cells expressing mutant htt and attenuated neurodegeneration in various models of neurodegenerative diseases. PPAR-γ-coactivator 1a (PGC-1 a) transcription activity is also impaired by mutant htt (Cui *et al*., 2006; Weydt et al., 2006), and represses genes targeted by PGC-1a in HD patients, as well as in HD mouse models (Chaturvedi et al., 2010). Over-expression of PGC-1a protects neurons from mutant htt-induced cell death, while PGC-1a knockout mice exhibited impaired mitochondrial dysfunction, movement disorders, and striatal degeneration (Chiang et al. 2010). PPAR-γ regulates glucose and lipid metabolism and suppress inflammatory gene expression (Landreth *et al*., 2008). PPAR-γ agonists have also been reported to effectively attenuate oxidative stress, inflammation and apoptosis in the central nervous system (CNS) (Kaundal and Sharma, 2010).

Antioxidant potential of NBV against oxidative stress is evident with lower levels of MDA and nitrite and higher levels of GSH. Nebivolol has been shown to have protective effects in models of experimental ischemia and reperfusion injury (Vandeplassche *et al*., 1991). One of important effects of this drug is the stimulation of formation and secretion of nitric monoxide (NO), the substance that is a key substance in prevention of the endothelial dysfunction that is crucial for the commencement of the atherogenic disorder (Kneaevi, 2008). Besides vasodilatation, NO has anti-aggregation effect, it reduces proliferation of smooth muscle cells and migration of leucocytes there by playing its role in inflammation (Brehm *et al*., 2000; Zanchetti, 2004; Wolf *et al*., 2008). Stimulation of PPARγ promotes mitochondrial biogenesis via the induction of the PGC-1α (Lehrke and Lazar, 2005). PPAR/PGC-1α pathway plays an important role in regulating cellular energy metabolism (Scarpulla, 2008). PGC-1α is also required for the induction of many ROS-detoxifying enzymes, including GPx1 and SOD2 (St-Pierre *et al*., 2006). It has been reported that nebivolol stimulates mitochondrial biogenesis (Huay *et al*., 2013). There are studies reporting that NBV administration results in reduction of amyloid β neuropathology in AD (Wang *et al*., 2013). Researchers have also reported NBV to be neuroprotective and potent antioxidant both on behavioural and microscopic levels (Nade *et al*., 2014; Manthey *et al*., 2010). NBV is reported to have antiepileptic activity along with antioxidative effects (Goel *et al*., 2009). Some of the published studie have reported that NBV is protective against dysleicsia and improves locomotor activity along with oxidative stress (Nade *et al*., 2013), also some of them reported NBV to be neuroprotective against ischemia reperfusion injury (Heeba and El-Hanagy, 2012).

Histopathological analysis represented that 3-NP treated rat brains showed severe degeneration, meningitis, haemorrhage and neuroinflammation by release of pro-inflammatory mediators in stratum and cortex area of cerebrum and purkinji cell degeneration in cerebellum. The animals pretreated with NBV (1mg/kg) showed positive changes in brain slides i.e less neuronal degeneration, less 2neuroinflammation in cerebrum and less purkinje degeneration in cerebellum as compared to NBV (0.5 mg/kg). Treatment with BADGE showed severe

neurodegeneration, neuroinflammation, haemorrhage, meningitis in cerebrum and purkinji cell degeneration in cerebellum as compared to vehicle treated brains even in presence of NBV 1 mg/kg).

Therefore with support from literature & data in hand, it may be suggested that nebivolol a β1-adrenergic antagonist and a proven antioxidant has shown ameliorative effects against 3-NP induced neurodegeneration as well as loss of learning & memory in rats. However, pretreatment with BADGE, a selective PPAR-γ antagonist abolished the nebivolol mediated protection that is signified by decrease in GSH, increase in AChE, MDA, MPO and nitrite levels when compared to high dose treated animals.

Hence, it is put forward that nebivolol provides neuroprotection against 3-NP induced HD in rats and this protection is mediated via activation of PPAR-γ receptors.

CONCLUSION:

The present study was designed to investigate the effects of Nebivolol in Wistar rats. 3-Nitropropionic acid at the dose of 10 mg/kg for 14 days was injected to the experimental animals to develop cognitive impairment. MWM was employed to test the learning and memory of animals. Biochemical estimations such as AChE activity, TBARS, GSH levels, MPO activity and nitrite/ nitrate levels was carried out. Brain total protein levels was also estimated. Histopathological studies were performed to ascertain the results cumulated from above studies.

The salient findings obtained in present study may be summarized as-

(1) Control group animals had shown a good performance on MWM test as reflected by normal learning ability and memory.

(2) Administration of Nebivolol (1 mg/kg; *p.o.*) *per se* had no effect on acquisition, learning and retrieval of memory using MWM as compared with control group.

(3) Administration of 3-Nitropropionic acid (10 mg/kg for 14 days, *i.p.*), impaired both learning and memory which is indicated by significantly decreased MWM performance. 3-NP caused signified decrease in body weight of animals and has shown significant increase in brain AChE activity, brain nitrite / nitrate levels, brain TBARS levels, and decrease in brain GSH levels.

(4) Treatment with Nebivolol (0.5 mg/kg and 1 mg/kg; *p.o.*) significantly attenuated 3-NP induced memory impairment along with a significant attenuation of increased serum glucose levels, nitrite/ nitrate levels, AChE activity, MPO activity and brain oxidative stress (decreased TBARS and increased GSH level).

(5) In addition, histopathological changes were also arrested. Pretreatment with BADGE abolished the neuroprotective effect of nebivolol on 3-NP treated animals.

Henceforth, on the basis of above findings it may be concluded that Nebivolol exert its protective effects on 3-NP induced huntington's disease by virtue of its PPAR-γ agonistic action being one of the prominent contributor.

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