Neuroprotective Effect of Filgrastim in Animal Models of 3-Nitropropionic Acid & Haloperidol Induced Neurotoxicity in Rats

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Research Article

Keywords: Huntington's Disease, Tardive dyskinesia, 3-nitropropionic acid, Haloperidol, Oxidative stress, Filgrastim

Posted Date: February 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-158354/v1

Abstract

Movement disorders are the heterogeneous group of disorders characterized by the progressive and selective impairment in motor function. Movement disorders like Huntington's disease (HD) and Tardive dyskinesia (TD) share many common features at both cellular and subcellular levels. Filgrastim is a recombinant methionyl granulocyte colony-stimulating factor (GCSF), shows neuroprotective properties in *in-vivo* models of movement disorders. The present study was designed to evaluate the neuroprotective effect of filgrastimin the animal models of haloperidol and 3-NP induced neurotoxicity in rats. Study was divided in two different protocol, in study one, rats were administered with haloperidol for 21 days, and filgrastim at the dose of (20, 40 & 60 µg/kg,*s.c.*) was administered once a day, before haloperidol treatment and the following parameters (orofacial movements, rotarod, actophotometer) were assessed for TD. Similarly, in second study rats were administered with 3-NP for 21 days and filgrastimat the dose of (20 & 40 µg/kg, *s.c.*) was administered, and following parameters (rotarod, narrow beam walk and open field test) were assessed for HD. In each study, on 22nd day, animals were sacrificed, to isolate cortex and striatum for oxidative stress (LPO, GSH, SOD, catalase, and nitrate) parameters. The result revealed that haloperidol and 3-NP treatment significantly impaired motor coordination, oxidative defense and induce TD and HD like symptoms. Filgrastim pre-treatment significantly averted haloperidol & 3-NP induced behavioral and biochemical alterations, respectively. Conclusively, neuroprotective effect of filgrastim is credited to its antioxidant properties, hence filgrastim might be a novel therapeutic candidate to manage TD & HD.

1. Introduction

Movement disorders, especially TD and HD consist of multiple pathophysiological similarities having similar kinds of locomotor manifestations. The intrinsic pathophysiology of both diseases is associated with neuronal death events by analogous pathways and show gate abnormalities. TD & HD both are hyperkinetic movement disorders in which the direct as well as in direct pathways play vital role in altering motor responses. Evidences reveal, altered expression of neurotransmitters makes under-activation of indirect pathways, is the most prominent pathophysiological feature of both disorders (Stahl, 2017; Starr et al., 2008). In the loop of indirect pathway, neurons that projected from striatum to globus pallidus interna (GPi) and globus pallidus externa (GPe) effects brutally leads to the loss of inhibition of GPe. Elevated activity of GPe, inhibit subthalamic nucleus (STN), substantia nigra pars reticulata (SNpr), and GPi, results in loss of inhibition of thalamus hence, increase thalamocortical excitatory drive and develop hyperkinetic movement abnormality (Schroll & Hamker, 2016). Furtermore, at cellular level, dopaminergic manifestations in both disorders induce glutamate mediated excitotoxic neuronal cell death (Chen et al., 2013). Evidences reveal, overactivation of NMDA receptors through NR2B subunit is responsible for the glutamatergic excitotoxicity in striatum. TD & HD brain consist of high free radical production, reduced ATP generation, which further impair Na+-K+-ATPase pump (normally maintain electronic gradients across cellular membranes) (Lerner et al., 2015; Prentice et al., 2015). This

results in to opening of NMDA receptors for linger period of time results in large influx of Ca^{2+} and generation of free radical and oxidative damage (Minnella et al., 2018).

Being a typical anti-psychotic haloperidol (HP) have a strong affinity to bind with dopamine D_2 receptor. This mechanism causes supersensitivity in the postsynaptic striatal dopamine D_2 receptors, influence MSN in indirect pathway, causing a wide range of side effects (facial jerking, vacuous chewing movements (VCM), cheek puffing, eye blinking, tongue protrusion, and lip-smacking) and likely contributes to the repetitive involuntary (orofacial) movement and develops TD (Lockwood & Remington, 2015; Thakur et al., 2015).

3-NP administration at the dose of 10mg/kg/*s.c.* in chronic fashion makes a neuronal loss in the striatum by inhibiting mitochondrial complex-II which increase the production of NO, oxidative stress and excitotoxicity, sensitization of NMDA results in the selective degeneration of GABAergic MSNs, impair indirect pathway and develop choreaform movement similarly as observed in HD patients (Brouillet, 2014; Khan et al., 2015). Followed by complex II inhibition 3-NP makes mitochondrial dysfunction by influencing mitochondrial dynamics and organelle trafficking, results in bioenergetic failure. Mitochondrial dysfunction is the most prominent feature of movement disorders like Huntington's Chorea, developed in response to 3-NP induced impairment in mitochondrial Ca^{2+} handling, transcription abnormalities, ATP production, electron transport chain (ETC) impairment (Brouillet, 2014; Kaur et al., 2015). **(Fig 1.1)**

From the last two-decade large number of therapeutic candidate has been proposed as a treatment strategy for movement disorders, especially for TD & HD but there inappropriate pharmacokinetic and toxicity profile hampers their protective effect. Presently available treatment approaches are based upon symptomatic relief; however, none of the treatment targets the ongoing neurodegenerative, which halt or delay the disease progression. Therefore, high weightage has been given to explore preexisted therapy, which is already available in the market for different clinical use, to save the time and money in initial preclinical studies. Moreover, apart from influencing the clinical outcome, such therapy reduces the chances of drug failure due to safety issues.

Filgrastim, a recombinant form of granulocyte colony-stimulating factor (G-CSF), use for neutropenia during chemotherapy. Filgrastim is highlighted as neuronal molecule when its subcutaneous administration cross BBB (Azmy et al., 2018). Filgrastim potentially inhibit glutamate induced neuronal cell dath considered as hallmark in both TD and HD (Pan et al., 2010). Both HP and 3NP significantly elevate the lipid peroxidaiton and nitrite level in brain and decrease the antioxidant levels (Naidu et al., 2003; Silva et al., 2007). However, filgrastim enhance the GSH level and repression of elevated MDA, NO, proinflammatory cytokines (El-Esawy et al., 2018). Multiple studies reflect the neuroregenerative and neuroprotective properties of GCSF in various animal models (Parkinson's disease, Alzheimer's disease, intracerebral hemorrhage, intracerebral hemorrhage, experimental allergic encephalomyelitis, spinal cord injury), creadeted to its antiapoptotic and antioxidant profile (Azmy et al., 2018; Koda et al., 2007; Peng, 2017; Qaribi et al., 2018; Tsai et al., 2007).

Accordingly, this study aimed to assess the potential role of filgrastim to counteract the effects of HP & 3-NP induced alteration in behavioral & biochemical levels in rats.

2. Material And Methods

2.1 Experimental animals

Rats (either sex) having 200-250 g of weight attained from the central animal house of Maharaja Ranjit Singh Punjab Technical University, Bathinda, Punjab (India) were used. The experimental animals were kept and handled in accordance with the follow up of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms, according to which polyacrylic cages were used to accommodate the animals with standard laboratory conditions which include room temperature 22 ± 2 C, relative humidity of 55- 60 % and 12-h light/dark cycle were maintained. Experimental feed pallet and water made available *ad libitum*. Entire behavioral studies were conducted between 9:00 am to 5:00 pm. The study was approved in two different protocols with reference no. MRSPTU/IAEC/2018/010 (study-1) and MRSPTU/IAEC/2019/09 (study-2) by the Institutional Animal Ethics Committee (IAEC). To avoid variability between experimental groups, aged match animals were used in all experiments for a given treatment.

2.2 Experimental groups

The assigned set animals were divided randomly in the treatment groups. Study-1 contain six groups and study-2 contain five groups, having six animals (n = 6) in each treatment group. **(Table 2.1 & 2.2)**.

2.3 Drugs and chemicals

3-NP was procured from Sigma Aldrich Chemicals Pvt Ltd, Banglore (India); filgrastim (Dr. Reddy's) and Haloperidol (Serenace® Inj., Searle India, and India) were purchased from the local market.

2.4 Treatment schedule

*Study 1:Haloperidol induced TD: -*Treatment schedule was synchronized in order to evoke TD like feature where the dose of 1 mg/kg Haloperidol administered intraperitoneally (*i.p.*) daily for 21 days by dissolving in buffered saline of (pH 7.4).Filgrastim was diluted with normal saline (N.S) and administered at a dose of (20, 40 & 60 µg/kg) subcutaneously (*s.c.*) 6 hours prior to haloperidol treatment. Weekly assessment of behavioral parameters like vacuous chewing movements (VCM), facial jerking (FJ), tongue protrusions (TP), rotarod, and actophotometer were accessed (1^{st}, 7^{th}, 14^{th}, and 21^{st} day) along with their body weights. Striatum and cortex were isolated after sacrifice on day 22 to estimate biochemical parameters include, lipid peroxidation (LPO), reduced glutathione (GSH), nitrite, catalase, protein estimation, and superoxide dismutase (SOD).

*Study 2: 3-NP induced HD: -*Treatment schedule was planned to evoke HD like feature,in which the dose of 10 mg/kg 3-NP administered intraperitoneally (*i.p.*) daily for 21 days by dissolving in buffered saline of

(pH 7.4). Filgrastim is used similarly to study 1. Weekly assessment of behavioral (rotarod, open field test, narrow beam walk) and biochemical (LPO, GSH, nitrite, catalase, protein, and SOD) parameters were accessed similarly to study 1.

2.5 Measurement of body weight

The percentage change in body weight was calculated on the behalf of the initial and final reading of the body weights, recorded at the 1st and 21st day, respectively before and after haloperidol (study 1) and 3-NP (study 2) treatment (Jamwal & Kumar, 2019).

Change in bodyweight = bodyweight (1st day−21st day) / 1st day body weight × 100.

2.6 Assessment of behavioral activity

2.6.1 Study 1: Haloperidol induced TD

2.6.1.1 ***Assessment of orofacial movements:*** On the test day (on 7th, 14th, and 21st day), each rat was gently placed in a small plexiglass cage (30×20×30 cm) for the assessment of oral dyskinesia. Animals were given 10 min to get acclimatized to the observation cage before behavioral assessments. To quantify assessment, the occurrence of oral dyskinesia, hand-operated counters were employed to score facial jerking (FJs), tongue protrusion (TPs), and vacuous chewing movements (VCMs). The behavioral parameters of oral dyskinesia were measured continuously for 10 min. In all the experiments, the scorer was unaware of the treatment given to the animals (Datta et al., 2016).

2.6.1.2 ***Rotarod test*****:** To evaluate the motor coordination and integrity of the animal rotarod test was performed. The constant speed of 25 rpm was maintained on the apparatus (IMCORP, Ambala, India) having length 30 cm and diameter 7 cm. Fall off latency was measured during the experiment with a cutoff time of 180 (Jamwal et al., 2017).

2.6.1.3 ***Locomotor activity:*** The locomotor activity of the experimental rat was monitored using activity meter (IMCORP, Ambala, India). Before subjecting the animal to the cognitive task, they were individually placed in activity meter, and the total activity count (upper beam and lower beam) was registered for 5 min. The locomotor activity was expressed in terms of total photo beams, counts/5 min per animal (Bishnoi et al., 2008).

2.6.2 Study 2: 3-NP induced HD like symptoms

2.6.2.1 ***Narrow beam walking test*****:** The apparatus comprises of two different platforms (8 cm in diameter) each side of the beam. The beam of 0.5 mm in thickness and 2.0 cm in width and 120 cm in length is placed 1 m above the ground. Sawdust box was placed below the beam to create a cushion effect to fall off the rat. Animals were acclimatized for 5 min. before the training and mean experiment time consist of 120 sec. where the time taken to cross the beam and the number of foot slips has been

recorded for each trial. The average of three measured transfer latency yield the final value with the interval of 2 min of inter trial (Jamwal & Kumar, 2016a; Khan et al., 2015).

2.6.2.2 ***Open Field Test (video tracking system):*** The behavior of all animals were captured at the end of the study using a video camera (VJ instruments, India) positioned above the square box (open field), comprises of a wooden rectangular black colored apparatus having dimension 61 × 61 × 40 cm. The floor of the apparatus was completely black to avoid any sensitivity issues during tracking. The experimental room was completely black, and an artificial light source was provided to support the video tracking system.To evaluate the locomotor activity, animals were gently placed wooden boxes (OFT), with 36 houses squares. Animals were given 5 min to get acclimatized and allowed to explore the area before behavioral assessments. The exploration in the open field, i.e., ambulation (the number of squares crossed with all paws), distance moved, and time spent moving (active time), time spent without movement (passive time), average speed wasrecorded by a video tracking system (VJ instruments, India) (Aswar et al., 2017). The three times measured activity during the experiment were averaged to produce a final value, and the cut-off time was 10 minutes (fig 2.1). After exposureto area for 10 min, rats were fear conditioned in the area (A square wooden chamber) that were cleaned with 70% ethanol before each session. In all the experiments, the scorer was unaware of the treatment given to the animals (Sivanathan et al., 2015; Zurn et al., 2005). **(Fig 2.1)**

2.6.2.3 ***Rotarod test*****:** Similar to study 1.

2.7 Dissection and Homogenization

Animals were sacrificed by cervical dislocation on 22nd day to isolate striatum/cortex for biochemical estimations. Tissue homogenate was prepared in accordance with 10% (wv-1) in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000×g for 20 min. The supernatant was separated for biochemical estimation.

2.8 Measurement of Oxidative Stress Parameters

2.8.1 *Measurement of lipid peroxidation*

In order to make a quantitative estimation of the extent of lipid peroxidation in the striatum and cortex of the brain the method proposed by Wills is used (Wills, 1966). Optical density was recorded 532 nm (Shimadzu spectrophotometer).

2.8.2 *Estimation of Nitrite*

Colorimetric assay is used for nitrite estimation where nitric oxide is produced in response to nitrite accumulation in the supernatant of striatum which wasestimated by Greiss reagent (0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride, 1 % sulfanilamide, and 2.5 % phosphoric acid) method described as (Green et al., 1982).

2.8.3 *Estimation of glutathione levels*

To estimate reduced glutathione (GSH)in the brain was estimated using Ellman method, (Ellman, 1959).

2.8.4 *Protein estimation*

Lowry method was used for the measurement of protein by using Folin phenol as reagent (Gornall et al., 1949).

2.8.5 *Catalase activity*

Catalase activity was measured according to the method described by (Luck, 1963) and change in absorbance at 240 nm for 2 min interval with 30/60 sec intervals using Shimadzu UV/visible spectrophotometer

2.8.6 *Superoxide dismutase (SOD) activity*

Superoxide dismutase activity was measured by the proposed method described as (Kono, 1978).

2.9 Statistical analysis

Values are expressed with means ± SD. The behavioral assessment data were analyzed using a two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test for multiple comparisons. For biochemical parameters, a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for comparison. $p<0.05$ was considered statistically significant.

3. Results

3.1 Study 1: Haloperidol induced TD

3.1.1 *Effect of Filgrastim on body weight in HP treated rats*

Chronic treated with haloperidol (1mg/kg, *i.p.*) for 21 days has not decrease body weight as compared to the vehicle treated group. The administration of filgrastim (20, 40, and 60 µg/kg, *s.c.*) along with haloperidol and filgrastim *per se* (60 µg/kg) not showed any significant effect on body weight as compared to haloperidol alone treated group. **(Fig 3.1.1)**

3.1.2 *Effect of filgrastim on HP induced orofacial movements*

Chronic administration of haloperidol at a dose (1mg/kg, *i.p.*) for 21 days results in a time dependent significantly ($p<0.001$) increase in vacuous chewing movements, facial jerking, and tongue protrusion compared to control-treated group. Pretreatment with filgrastim (40 and 60 g/kg,*s.c.*) significantly reverse haloperidol-induced vacuous chewing movements, facial jerking, and tongue protrusion as compared to respective haloperidol treated group. Whereas filgrastim (20 µg/kg, *s.c.*) failed to reverse in haloperidol-induced orofacial movements, and filgrastim *per se* group had no effect. **(Fig. 3.1.2; 3.1.3; 3.1.4)**

3.1.3 *Effect of filgrastim on HP induced changes in rotarod activity.*

Systemic HP (1mg/kg, *i.p.*) treatment produced significant ($p<0.01$) gradual decrease in rotarod activity as compared to the control group**.** Pretreatment with filgrastim (40 and 60 µg/kg, *s.c.*) significantly ($p<0.05$) prevented the impairment in rotarod activity as compared to haloperidol treated rats. However, treatment with a lower dose (20 µg/kg, *s.c.*) does not have any significant effect on rotarod activity. Filgrastim *per se* group did not affect. **(Fig 3.1.5)**

3.1.4 *Effect of filgrastimin locomotor activity (actophotometer) in HP treated rats.*

Chronic treatment with haloperidol (1mg/kg, *i.p.*) for 21 days induced significant ($p<0.001$) decrease in locomotor activity as compared to the respective control-treated group. Pretreatment with filgrastim 20 µg/kg showed no effect, but the pretreatment with filgrastim (40 and 60 µg/kg, *s.c.*) significantly ($p<0.05$) reversed the haloperidol-induced alteration of locomotor activity day as compared to respective haloperidol treated group. Further, the administration of filgrastim *per se* has showed no effect. **(Fig 3.1.6)**

3.1.5 *Effect of filgrastim on oxidative stress in HP treated rats*

Chronic treatment with haloperidol at a dose of 1 mg/kg, *i.p.* for 21 days, increase in MDA and nitrite level and decrease in GSH, SOD, and catalase level in comparison with control animals ($P<0.001$). Filgrastim (40& 60 µg/kg, *s.c.*) treatment produces a marked improvement in 3-NP induced increase in oxido-nitrosative stress ($P<0.05$). **(Table 3.1.1)**

3.2 Study 2: 3-NP induced HD like symptoms

3.2.1 *Effect of Filgrastim on body weight in 3-NP treated rats*

No change is observed in the initial and final bodyweight of the animal in the control group, whereas significant ($P<0.05$) decreased in body weight (% change in body weight) in 3-NP treated animals is observed when compared with the control group on day 21. Filgrastim (20, 40, µg/kg, *s.c.*) treatment in 3-NP treated rats significantly ($P<0.05$) attenuated the decrease in body weight (Fig. 5.1). Filgrastim *per se* (40 mg/kg, *s.c.*) treatment does not possess any significant effect on body weight. **(Fig 3.2.1)**

3.2.2 *Effect of filgrastim in time to cross narrow beam and no. of foot slips on narrow beam walking apparatus in 3-NP treated rats.*

3-NP administered systemically (10 mg/kg; *i.p.*) produces significant ($P<0.001$) increased in the time taken to cross across the beam and number of slips in comparison to the control group ($p < 0.001$) on 21st day. Filgrastim *per se* group did not show any effect. Further, pre-treatment with filgrastim (20 & 40 µg/kg, *s.c.*) significantly ($P<0.05$) decreased the number of slips, time taken to cross the beam, and improved the gait abnormalities as compared to 3-NP group. **(Fig 3.2.2A; 3.2.2B)**

3.2.3 *Effect of filgrastim on 3-NP induced changes in rotarod activity.*

Systemic 3-NP (10 mg/kg/day; i.p) treatment produced significant ($p<0.01$) gradual decrease in rotarod activity on day 14^{th} and day 21^{st} as compared to control group**.** Pretreatment with higher dose of filgrastim i.e. (40 µg/kg, s.c) significantly ($p<0.05$) prevented the impairment in rotarod activity as compared to 3-NP treated rats. However, treatment with lower dose (20 µg/kg, s.c) do not have any significant effect on rotarod activity. Filgrastim *per se* group had no effect. **(Fig 3.2.3)**

3.2.4 *Effect of filgrastim on motor coordination Open Field Test (OFT) in 3-NP treated rats.*

The video tracking software system (Maze Master 3.0) evaluates the activity of 3-NP treated animals, shows a significant decrease in the number of crossing, active time, average speed, total distance, and increase passive time on 7th, 14th, and 21st days as compared to control group. Whereas pretreatment with filgrastim (20 & 40 µg/kg, *s.c.*) significantly ($P<0.05$) reversed the 3-NP induced behavioral alteration. Furthermore, filgrastim *per se* group did not affectas compared to the normal group **(Fig 3.2.4; 3.2.5; 3.2.6; 3.2.7; 3.2.8).**

3.2.5 *Effect of filgrastim on oxidative stress in 3-NP treated rats.*

The 3-NP treatment produces a considerable increase in oxido-nitrosative stress i.e., increase in MDA and nitrite level and decrease in GSH, SOD, and catalase level in comparison with normal control animals ($P<0.001$). Filgrastim (20 &40 µg/kg,*s.c.*) treatment produces a marked improvement in 3-NP induced increase in oxido-nitrosative stress ($P<0.05$). **(Table 3.2.1)**

4. Discussion

In the present study, HP & 3-NP induce multiple systemic metabolic alterations and reflects the pathophysiological feature of TD and HD respectively. Administration of these agents for 21 days elicit sustained metabolic alterations evoked in the form of a stable motor deficit in each study. Haloperidol evokes orofacial involuntary movements such as VCM's, facial jerking, and tongue protrusion along with impairments in locomotory function expressed as a decrease in rotarod test and actophotometer activity. Whereas the 3-NP show decrease in body weight, fall-off time in a rotarod test, elevation in the time taken to cross the beam (transfer latency) and no. of footslips in narrow beam walk and impairment in locomotor activity accessed by the different parameters of open field test such as a decrease in a number of crossing, active time, average speed, total distance and increase in passive time, thus representing sustained motor deficit and striatal degeneration. Furthermore, biochemical consequences initiated after HP & 3-NP challenge in each study which includes lipid peroxidation associated with oxidative stress perceive the formation of malondialdehyde (MDA) and elevate the nitrite level in the rat brain, whereas, decrease in the level of antioxidant enzymes (SOD, catalase, GSH) is observed, which are in agreement with the previous studies (Datta et al., 2016; Jamwal & Kumar, 2016b).

Haloperidol block striatal D2 receptor resulting produced supersensitivity in the postsynaptic striatal dopamine D2 receptor (Servonnet & Samaha, 2019). Chronic administration of neuroleptics like haloperidol increase the striatal release of glutamate from the cortico-striatal terminals, leading to striatal

excitotoxicity, hyperactivation of the NMDA receptors due to the loss of inhibition of voltage-dependent Mg^{2+}, and physiological levels of glutamate then triggers the excessive influx calcium (Zhuravliova et al., 2007) and excitatory dopamine receptor pathway is upregulated corresponding to hyperkinetic dyskinetic movements originating from the striatum. Proliferating inhibitory D2 receptors with chronic treatment of haloperidol increases excessive free radicals production, which is indicative of increased lipid peroxidation and nitrite level in the brain and decreased antioxidant defensive mechanisms (Bishnoi et al., 2008). Chronic neuroleptics administration leads to alteration of the production and detoxification of free radicals and increased permeability of various membranes, which may be associated with the initiate the contribution of repetitive, involuntary orofacial movements (Wu et al. 2014). Long time administration of haloperidol increases in the turnover of dopamine, which may lead to the formation of ROS and responsible for oxidative stress (Polydoro et al., 2004). All these effects of haloperidol are in good agreement with previous studies (Bishnoi et al., 2008; Datta et al., 2016).

3-NP a mitochondrial toxin mimics the spectrum of HD-like features in the striatum of rat upon systemic administration and hence represents itself as a gold standard model to induce of HD like symptoms (Brouillet, 2014; Khan et al., 2015). The neuro-pathophysiological feature of 3-NP is associated with the generation of free radicals and oxidative stress by altering the multiple cellular pathways. The irreversible inhibition of respiratory chain complex II is the most prominent feature that contributes to 3-NP toxicity. The data from the previous reports reflects the systemic profile of 3-NP, according to which 3-NP induced complex II inhibition stimulates the reactive oxygen species (ROS) production followed by alteration in multiple intercellular events, including ATP and energy failure, leads to mitochondrial dysfunction (Brouillet, 2014). At mitochondrial level, 3-NP induced enzymatic inhibition include an increase in electrons released from the mitochondria and the subsequent production of ROS leads to increase lipid peroxidation and nitrite level and decrease antioxidant levels (SOD, catalase, GSH) in brain (Singh et al., 2015).

Furthermore, 3-NP induced elevation in the production of NO, oxidative stress, and excitotoxicity by sensitization of NMDA,which show preferential degeneration of the GABA-ergic MSNs in the striatum (Brouillet, 2014). Such brutally affected the MSNs impair the balance between the direct and indirect pathway in the striatum and develop choreiform movements and other behavioral manifestations (Brouillet, 2014; Khan et al., 2015). Treatment with 3-NP trigger unintended weight loss and movement abnormalities, is a hallmark of HD, but our present knowledge is not sufficient enough to evaluate the rate of disease progression based on extent weight loss (Aziz et al., 2008). The 3-NP model elicits time and dose-dependent actions by developing hyperkinetic and hypokinetic symptoms, respectively, which further allow the assessment of the initial (or early) and late phases of HD (Túnez et al., 2010).

The present study highlights the neuronal moiety, which not only fills the void of neuroprotection but potent enough to be immediately transferred to clinical studies. Along with its active use, filgrastim (a recombinant form of GCSF) possesses an excellent clinical reputation in concern with the safety profile for neutropenia patients (Crawford, 2003). Along with its constructive properties to manage survival, differentiation, and proliferation of hematopoietic system (Mangan & Reddy, 2005; Meuer et al., 2006),

filgrastim show direct anti-apoptotic action in hematopoietic cells which is appeared to be preserved well in the neuronal system (Schneider et al., 2005). Moreover, apart from its commendable therapeutic performance in the model of cerebral ischemia, Alzheimer's disease, and Parkinson's disease, filgrastim emerge as a new hope in order to treat movement disorders, like HD & TD. Importantly, its favorable pharmacokinetic profile includes its availability to the brain by penetrating the intact blood-brain barrier (Schneider et al., 2005). Furthermore, this molecule possesses the ability to inhibit glutamate-induced excitotoxicity, which is the key pathophysiological feature of both HD & TD (Pan et al., 2010).

Administration of filgrastim attenuate HP & 3-NP induced locomotor impairment. As already known that systemic administration of HP & 3-NP induces striatal lesions in rats and impair locomotor activity in rats (Andreassen et al., 1998; Brouillet, 2014). Locomotory impairments and motor deficits are associated with HP & 3-NP induced excitotoxicity and selective degeneration of GABAergic MSNs of the striatum, whereas the data from previous studies reflect the neuroprotective profile GCSF (filgrastim) to prevent neuronal cell death specially MSNs in the striatum (Kutlu et al., 2018; Lee et al., 2008). Moreover, GCSF administration can improve cognitive impairment in the experimental model of AD and PD (Azmy et al., 2018; Sanchez-Ramos et al., 2012), which further confirms its protective role in locomotory impairments. All these studies favor the protective potential of filgrastim against HP & 3-NP induced motor deficits.

Filgrastim attenuate biochemical levels by upregulating bcl-2 and modulating the cellular function of STAT protein, which ultimately contributes to an increase in the activity of antioxidant defense mechanism, verified by both elevation in activity of antioxidant enzymes as well as incremental availability of GSH (Lee et al., 2008). Whereas coadministration of filgrastim with HP (study-1) and 3-NP (study-2), makes significant improvisation in the activity of antioxidant enzymes such as catalase, SOD, and GSH, which further underline the potent antioxidant property of filgrastim. G-CSF can effectively prevent mitochondrial swelling, mitochondrial membrane potential changes, and ROS production in mitochondria. Further, it rescues the cells from oxidative stress-induced apoptosis, hence potentially combat HP and 3-NP induced cellular manifestations (Kojima et al., 2011). Our present data is in good agreement with previous observation reveals free radical quenching property and an increase in antioxidant enzymes, which established a base to the neuroprotective aspect of filgrastim (Azmy et al., 2018).

Filgrastim possesses the ability to bind GCSFR after penetrating BBB (Zhao et al., 2007). Data from the previous study represent the expression GCSFR in the striatal region of the brain (Lee et al., 2008). The same group claims the GSCF activates signaling pathways, including ERK, Akt, and STAT, which are effective survival pathways that result in neuroprotection by triggering its anti-apoptotic profile (Lee et al., 2008). In the present study, treatment with filgrastim provoke improvement in motor coordination, locomotion activity, and biochemical levels, which is relatable to antioxidants as well as the anti-apoptotic property of filgrastim and hence show neuroprotective action in striatal region.

Various researcher around the globe is continuously working on the different growth factors as well as on their recombinant forms to develop a better therapeutic moiety. Although, specifically for TD & HD

number of growth factors has been studies previously which include BDNF, FGF, erythropoietin (EPO) and CNTF apart from their active profiles these growth factors have not succeeded yet due to specific limitation such as preclinical ineffectiveness (Gil et al., 2004), side effects upon repeated administration (La Spada, 2005) and issues regarding drug delivery to CNS (Shyu et al., 2006). But in contrast to this filgrastim potentially overcome all these limitatinos and represent itself as a better therapeutic agent by potentially attenuating HP & 3-NP induced impairment, gate abnormalities and biochemical levels in brain. Hence, it can be concluded as that filgrastim as an active moiety already in therapeutic use for other disease, having approved safety profile and its effects studied on CNS seems to be clinically real.

Declarations

Ethical Approval

Not applicable to this research article.

Consent to Participate

PK conceived and design of research. **VR** conducted the experiment on 3-NP induced Huntington's like symptoms and **PR** conducted the experiment on haloperidol induced Tardive Dyskinesia and **PK, VR** and **PR** wrote the manuscript. All author read and approve the manuscript and all data were generated in-house and that no paper mill was used.

Consent to Publish

All authors are agreed to publish the paper in this journal.

Author's contribution

PK conceived and design of research. **VR** conducted the experiment on 3-NP induced Huntington's like symptoms and **PR** conducted the experiment on haloperidol induced Tardive Dyskinesia and **PK, VR** and **PR** wrote the manuscript. All author read and approve the manuscript and all data were generated in-house and that no paper mill was used.

Funding

No funding received.

Competing Interests

Authors have no conflict of interest.

Availability of data and materials

Data and material will be available as suppliment data.

References

1. Andreassen OA, Ferrante RJ, Beal MF & Jørgensen HA (1998) Oral dyskinesias and striatal lesions in rats after long-term co-treatment with haloperidol and 3-nitropropionic acid. Neuroscience 87(3):639–648.
2. Aswar U, Chepurwar S, Shintre S & Aswar M (2017) Telmisartan attenuates diabetes induced depression in rats. Pharmacological Reports 69(2):358–364.
3. Aziz NA, Van Der Burg JMM, Landwehrmeyer GB, Brundin P, Stijnen T, Roos RAC & Group ES (2008) Weight loss in Huntington disease increases with higher CAG repeat number. Neurology 71(19):1506–1513.
4. Azmy MS, Menze ET, El-Naga RN & Tadros MG (2018) Neuroprotective Effects of Filgrastim in Rotenone-Induced Parkinson's Disease in Rats: Insights into its Anti-Inflammatory, Neurotrophic, and Antiapoptotic Effects. Molecular Neurobiology 55(8):6572–6588.
5. Bishnoi M, Chopra K & Kulkarni SK (2008) Protective effect of Curcumin, the active principle of turmeric (Curcuma longa) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. Pharmacology Biochemistry and Behavior 88(4):511–522.
6. Brouillet E (2014) The 3-NP model of striatal neurodegeneration. Current Protocols in Neuroscience 67(1):9–48.
7. Chen JY, Wang EA, Cepeda C & Levine MS (2013) Dopamine imbalance in Huntington's disease: a mechanism for the lack of behavioral flexibility. Frontiers in Neuroscience 7114.
8. Crawford J (2003) Safety and efficacy of pegfilgrastim in patients receiving myelosuppressive chemotherapy. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 23(8P2):15S-19S.
9. Datta S, Jamwal S, Deshmukh R & Kumar P (2016) Beneficial effects of lycopene against haloperidol induced orofacial dyskinesia in rats: possible neurotransmitters and neuroinflammation modulation. European Journal of Pharmacology 771229–235.
10. El-Esawy R, Balaha M, Kandeel S, Hadya S & El-Rahman M-NA (2018) Filgrastim (G-CSF) ameliorates Parkinsonism l-dopa therapy's drawbacks in mice. Basal Ganglia 1317–26.
11. Ellman GL (1959) Archs: Tissue sulfhydril groups. Biochem Biophys 8270–77.
12. Gil JMAC, Leist M, Popovic N, Brundin P & Petersén Å (2004) Asialoerythropoetin is not effective in the R6/2 line of Huntington's disease mice. BMC Neuroscience 5(1):17.
13. Gornall AG, Bardawill CJ & David MM (1949) Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry 177(2):751–766.
14. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS & Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Analytical Biochemistry 126(1):131–138.
15. Jamwal S, & Kumar P (2016) (a) Physiology & Behavior Spermidine ameliorates 3-nitropropionic acid (3-NP) -induced striatal toxicity : Possible role of oxidative stress , neuroin fl ammation , and

neurotransmitters. Physiology & Behavior 155180–187.

16. Jamwal S, & Kumar P (2016) (b) Spermidine ameliorates 3-nitropropionic acid (3-NP)-induced striatal toxicity: possible role of oxidative stress, neuroinflammation, and neurotransmitters. Physiology & Behavior 155180–187.
17. Jamwal S, & Kumar P (2019) Insight Into the Emerging Role of Striatal Neurotransmitters in the Pathophysiology of Parkinson's Disease and Huntington's Disease: A Review. Current Neuropharmacology 17(2):165–175.
18. Jamwal S, Singh S, Gill JS & Kumar P (2017) L-theanine prevent quinolinic acid induced motor deficit and striatal neurotoxicity: Reduction in oxido-nitrosative stress and restoration of striatal neurotransmitters level. European Journal of Pharmacology 811(January):171–179.
19. Kaur N, Jamwal S, Deshmukh R, Gauttam V & Kumar P (2015) Beneficial effect of rice bran extract against 3-nitropropionic acid induced experimental Huntington's disease in rats. Toxicology Reports 21222–1232.
20. Khan A, Jamwal S, Bijjem KR V, Prakash A & Kumar P (2015) Neuroprotective effect of hemeoxygenase-1/glycogen synthase kinase-3β modulators in 3-nitropropionic acid-induced neurotoxicity in rats. Neuroscience 28766–77.
21. Koda M, Nishio Y, Kamada T, Someya Y, Okawa A, Mori C, Yoshinaga K, Okada S, Moriya H & Yamazaki M (2007) Granulocyte colony-stimulating factor (G-CSF) mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice. Brain Research 1149223–231.
22. Kojima H, Otani A, Oishi A, Makiyama Y, Nakagawa S & Yoshimura N (2011) Granulocyte colony-stimulating factor attenuates oxidative stress–induced apoptosis in vascular endothelial cells and exhibits functional and morphologic protective effect in oxygen-induced retinopathy. Blood 117(3):1091–1100.
23. Kono Y (1978) Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics 186(1):189–195.
24. Kutlu MG, Brady LJ, Peck EG, Hofford RS, Yorgason JT, Siciliano CA, Kiraly DD & Calipari ES (2018) Granulocyte colony stimulating factor enhances reward learning through potentiation of mesolimbic dopamine system function. Journal of Neuroscience 38(41):8845–8859.
25. La Spada AR (2005) Huntington's disease and neurogenesis: FGF-2 to the rescue? Proceedings of the National Academy of Sciences 102(50):17889–17890.
26. Lee S-T, Park J-E, Kim D-H, Kim S, Im W-S, Kang L, Jung SH, Kim M-W, Chu K & Kim M (2008) Granulocyte-colony stimulating factor attenuates striatal degeneration with activating survival pathways in 3-nitropropionic acid model of Huntington's disease. Brain Research 1194130–137.
27. Lerner PP, Miodownik C & Lerner V (2015) Tardive dyskinesia (syndrome): current concept and modern approaches to its management. Psychiatry and Clinical Neurosciences 69(6):321–334.
28. Lockwood JT, & Remington G (2015) Emerging drugs for antipsychotic-induced tardive dyskinesia: investigational drugs in Phase II and Phase III clinical trials. Expert Opinion on Emerging Drugs

20(3):407–421.

29. Luck H (1963) Methods of enzymatic analysis. Enzyme Assays in Vivo. New York, London: Academic Press
30. Mangan J, & Reddy EP (2005) Activation of the Jak3 pathway and myeloid differentiation. Leukemia & Lymphoma 46(1):21–27.
31. Meuer K, Pitzer C, Teismann P, Krüger C, Göricke B, Laage R, Lingor P, Peters K, Schlachetzki JCM & Kobayashi K (2006) Granulocyte-colony stimulating factor is neuroprotective in a model of Parkinson's disease. Journal of Neurochemistry 97(3):675–686.
32. Minnella AM, Zhao JX, Jiang X, Jakobsen E, Lu F, Wu L, El-Benna J, Gray JA & Swanson RA (2018) Excitotoxic superoxide production and neuronal death require both ionotropic and non-ionotropic NMDA receptor signaling. Scientific Reports 8(1):1–13.
33. Naidu PS, Singh A & Kulkarni SK (2003) Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 44(8):1100–1106.
34. Pan C, Gupta A, Prentice H & Wu J-Y (2010) Protection of taurine and granulocyte colony-stimulating factor against excitotoxicity induced by glutamate in primary cortical neurons. Journal of Biomedical Science 17(S1):S18.
35. Peng W (2017) G-CSF treatment promotes apoptosis of autoreactive T cells to restrict the inflammatory cascade and accelerate recovery in experimental allergic encephalomyelitis. Experimental Neurology 28973–84.
36. Polydoro M, Schröder N, Lima MNM, Caldana F, Laranja DC, Bromberg E, Roesler R, Quevedo J, Moreira JCF & Dal-Pizzol F (2004) Haloperidol-and clozapine-induced oxidative stress in the rat brain. Pharmacology Biochemistry and Behavior 78(4):751–756.
37. Prentice H, Modi JP & Wu J-Y (2015) Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases. Oxidative Medicine and Cellular Longevity 2015
38. Qaribi M, Yazdanbakhsh A, Ahmadi K, Maghsoudi MR, Farshin V, Ahmadi A & Jokar A (2018) The effects of filgrastim on complications of patients with cerebral hemorrhage due to head trauma. Open Access Macedonian Journal of Medical Sciences 6(11):2030.
39. Sanchez-Ramos J, Cimino C, Avila R, Rowe A, Chen R, Whelan G, Lin X, Cao C & Ashok R (2012) Pilot study of granulocyte-colony stimulating factor for treatment of Alzheimer's disease. Journal of Alzheimer's Disease 31(4):843–855.
40. Schneider A, Krüger C, Steigleder T, Weber D, Pitzer C, Laage R, Aronowski J, Maurer MH, Gassler N & Mier W (2005) The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. The Journal of Clinical Investigation 115(8):2083–2098.
41. Schneider A, Krüger C, Steigleder T, Weber D, Pitzer C, Maurer M, Gassler N, Kollmar R, Schwab S & Kuhn H-G (2005) The hematopoietic factor G-CSF is a neuronal ligand that drives neurogenesis. Journal of Cerebral Blood Flow & Metabolism 25(1_suppl):S235–S235.

42. Schroll H, & Hamker FH (2016) Basal Ganglia dysfunctions in movement disorders: what can be learned from computational simulations. Movement Disorders 31(11):1591–1601.
43. Servonnet A, & Samaha A-N (2019) Antipsychotic-evoked dopamine supersensitivity. Neuropharmacology
44. Shyu W-C, Lin S-Z, Lee C-C, Liu DD & Li H (2006) Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. Cmaj 174(7):927–933.
45. Silva RH, Abílio VC, Kameda SR, Takatsu-Coleman AL, Carvalho RC, Ribeiro R de A, Tufik S & Frussa-Filho R (2007) Effects of 3-nitropropionic acid administration on memory and hippocampal lipid peroxidation in sleep-deprived mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry 31(1):65–70.
46. Singh S, Jamwal S & Kumar P (2015) Piperine enhances the protective effect of curcumin against 3-NP induced neurotoxicity: possible neurotransmitters modulation mechanism. Neurochemical Research 40(8):1758–1766.
47. Sivanathan S, Thavartnam K, Arif S, Elegino T & McGowan PO (2015) Chronic high fat feeding increases anxiety-like behaviour and reduces transcript abundance of glucocorticoid signalling genes in the hippocampus of female rats. Behavioural Brain Research 286265–270.
48. Stahl SM (2017) Neuronal traffic signals in tardive dyskinesia: not enough “stop” in the motor striatum. CNS Spectrums 22(6):427–434.
49. Starr PA, Kang GA, Heath S, Shimamoto S & Turner RS (2008) Pallidal neuronal discharge in Huntington’s disease: support for selective loss of striatal cells originating the indirect pathway. Experimental Neurology 211(1):227–233.
50. Thakur KS, Prakash A, Bisht R & Bansal PK (2015) Beneficial effect of candesartan and lisinopril against haloperidol-induced tardive dyskinesia in rat. Journal of the Renin-Angiotensin-Aldosterone System 16(4):917–929.
51. Tsai K-J, Tsai Y-C & Shen C-KJ (2007) G-CSF rescues the memory impairment of animal models of Alzheimer’s disease. The Journal of Experimental Medicine 204(6):1273–1280.
52. Túnez I, Tasset I & Santamaría A (2010) 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington’s disease: past, present and future. Molecules 15(2):878–916.
53. Wills E (1966) Mechanisms of lipid peroxide formation in animal tissues. Biochemical Journal 99(3):667.
54. Wu JQ, Tan YL, Tan SP, Wang ZR, Xiu MH, De Yang F & Zhang XY (2014) Cognition impairment in schizophrenia patients with tardive dyskinesia: association with plasma superoxide dismutase activity. Schizophrenia Research 152(1):210–216.
55. Zhao L-R, Navalitloha Y, Singhal S, Mehta J, Piao C-S, Guo W-P, Kessler JA & Groothuis DR (2007) Hematopoietic growth factors pass through the blood–brain barrier in intact rats. Experimental Neurology 204(2):569–573.
56. Zhuravliova E, Barbakadze T, Natsvlishvili N & Mikeladze DG (2007) Haloperidol induces neurotoxicity by the NMDA receptor downstream signaling pathway, alternative from glutamate

excitotoxicity. Neurochemistry International 50(7–8):976–982.

57. Zurn JB, Hohmann D, Dworkin SI & Motai Y (2005) A real-time rodent tracking system for both light and dark cycle behavior analysis. 2005 Seventh IEEE Workshops on Applications of Computer Vision (WACV/MOTION'05)-Volume 1 187–92.

Tables

Study 1: Haloperidol induced TD

GROUPS	TREATMENT	
1.	Vehicle treated group (Control)	n=6 M:F=3:3
2.	Haloperidol (1 mg/kg *i.p.*) for 21 days	
3.	Filgrastim (20 mg/kg *s.c.*) + Haloperidol (1 mg/kg *i.p.*) for 21 days	
4.	Filgrastim (40 mg/kg *s.c.*) + Haloperidol (1 mg/kg *i.p.*) for 21 days	
5.	Filgrastim (60 mg/kg *s.c.*) + Haloperidol (1 mg/kg *i.p.*) for 21 days	
6.	Filgrastim *Per se* (60 mg/kg *s.c*) for 21 days	

Table 2.1: Experimental Groups

Study 2: 3-NP induced HD like symptoms

GROUPS	TREATMENT	
1	Vehicle Control	n=6 M:F=3:3
2	3-NP (10 mg/kg/i.p/day) for 21 days	
3	Filgrastim (20 mg/kg/s.c) + 3-NP (10 mg/kg/i.p/day) for 21 days	
4	Filgrastim (40 mg/kg/s.c) + 3-NP (10 mg/kg/i.p/day) for 21 days	
5	Filgrastim *per se* (40 mg/kg/s.c)	

Table 2.2: Experimental Groups

Study 1: Haloperidol induced TD

Treat-ment group		MDA nmol/mg protein	Nitrite level mmol/mg Protein	GSH (µmol GSH/mg protein)	SOD unit/mg protein	Catalase (µmol of H_2O_2 decomposed/ min/mg protein)
Control	S	3.02±0.61	126.5±32.96	0.075±0.013	5.62±1.27	3.32±0.76
	C	2.44±0.46	96.91±17.69	0.069±0.009	4.72±0.54	2.94±0.38
HP	S	10.27±2.10 [a]	414.5±26.3 [a]	0.017±0.012 [a]	1.34±0.76 [a]	0.54±0.38 [a]
	C	10.34±1.59 [a]	366.1±44.1 [a]	0.014±0.004 [a]	0.92±0.95 [a]	0.58±0.44 [a]
FG(20)	S	9.27±1.25	382.2±19.5	0.023±0.004	1.73±0.86	0.84±0.47
	C	9.42±0.98	352.6±18.8	0.020±0.010	1.48±0.28	0.78±0.68
FG(40)	S	8.44±1.16 [b]	374.1±18.8	0.028±0.008 [b]	2.52±0.71	1.18±0.45
	C	8.27±0.70 [b]	344.5±44.1	0.031±0.006 [b]	2.06±0.95	1.06±0.48
FG(60)	S	7.89±0.65 [b]	339.2±22.8 [b]	0.034±0.007 [b]	2.78±0.68 [b]	1.32±0.53[b]
	C	7.96±0.95 [b]	285.3±57.4 [b]	0.037±0.008 [b]	2.45±0.46 [b]	1.32±0.51[b]
Per se	S	3.38±1.05	129.2±47.9	0.071±0.005	5.17±0.01	3.002±0.54
	C	2.93±0.93	126.5±23.7	0.068±0.007	4.34±0.47	2.59±0.33

Table 3.1.1: Values are expressed as Mean ± S.D. Data analyzed by one way ANOVA followed by Tukey's post hoc test.

[a]p< 0.001 vs. VC; [b]p< 0.05 vs. HP.

Note: S- Striatum; C- Cortex; HP- Haloperidol; FG- Filgrastim; VC- Vehicle control

Study 2: 3-NP induced HD like symptoms

Treat-ment group		MDA nmol/mg protein	Nitrite level mmol/mg Protein	GSH (µmol GSH/mg protein)	SOD unit/mg protein	Catalase (µmol of H_2O_2 decomposed/ min/mg protein)
Control	S	0.51±0.12	131.3±29.4	0.017±0.001	2.67±0.44	1.45±0.10
	C	0.37±0.09	111.8±26.9	0.016±0.002	2.57±0.43	1.31±0.09
3-NP	S	3.83±0.47 [a]	471.0±28.6 [a]	0.006±0.002 [a]	0.72±0.21 [a]	0.87±0.13 [a]
	C	3.76±0.28 [a]	435.4±29.7 [a]	0.009±0.002 [a]	0.39±0.13 [a]	0.75±0.10 [a]
FG(20)	S	2.91±0.28 [b]	393.3±19.5 [b]	0.010±0.002	1.36±0.31 [b]	1.06±0.09
	C	2.97±0.41 [b]	380.3±17.7 [b]	0.009±0.002	0.86±0.20 [b]	0.94±0.15
FG(40)	S	1.90±0.23 [b]	292.0±40.1 [b]	0.013±0.001 [b]	1.61±0.36 [b]	1.25±0.13 [b]
	C	2.01±0.29 [b]	287.4±23.1 [b]	0.013±0.002 [b]	1.57±0.20 [b]	1.16±0.14 [b]
Per se	S	0.64±0.09	140.6±15.7	0.017±0.001	2.52±0.29	1.43±0.07
	C	0.37±0.12	135.7±26.4	0.016±0.001	2.78±0.44	1.43±0.10

Table 3.2.1: Values are expressed as Mean ± S.D. Data analyzed by one-way ANOVA followed by Tukey's post hoc test.

[a]p< 0.001 vs. VC; [b]p< 0.05 vs. 3-NP.

Note: S- Striatum; C- Cortex; 3-NP- 3-nitropropionic acid; FG- Filgrastim; VC- Vehicle control

Figures

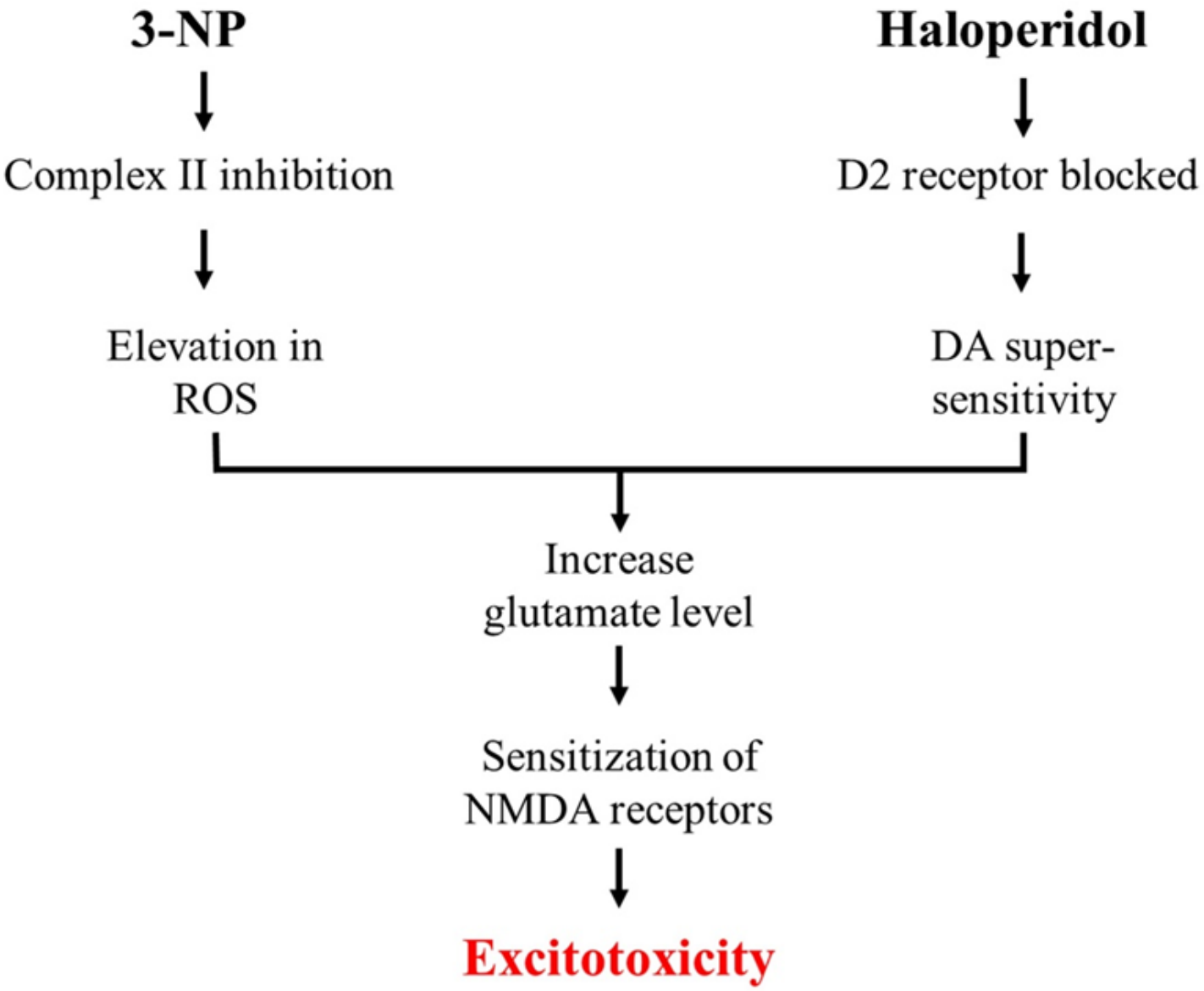

Figure 1

Pathophysiological mechanism behind excitotoxicity in HD and TD

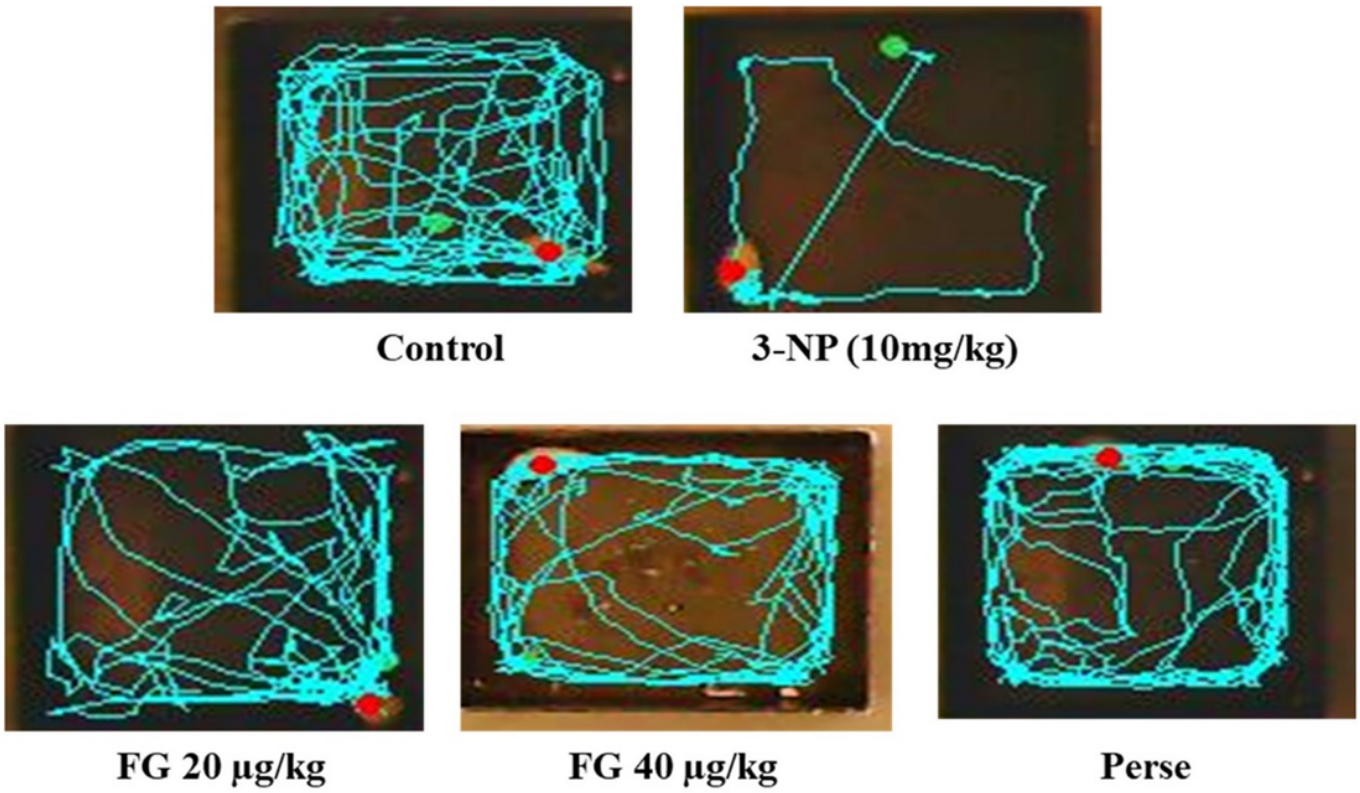

Figure 2

Open field test by video tracking system (Maze Master 3.0)

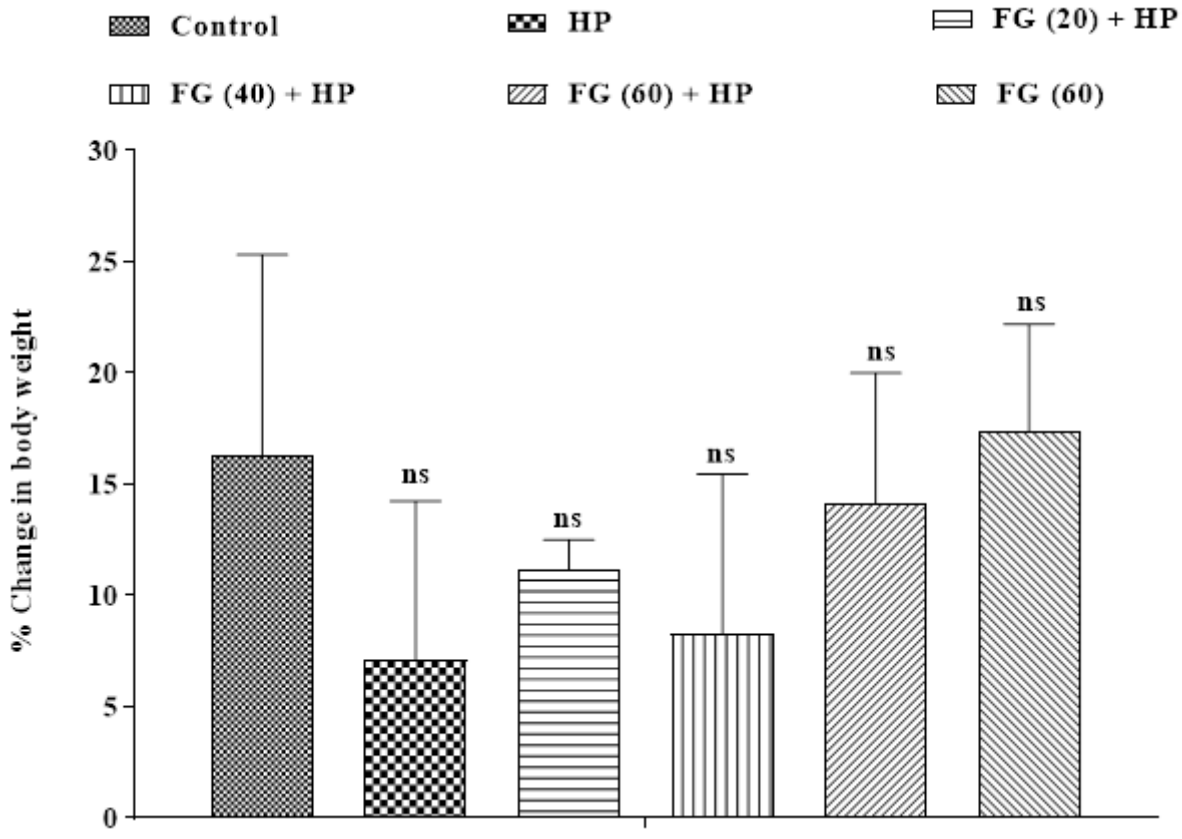

Figure 3

Haloperidol induced TD Effect of filgrastim on body weight in haloperidol treated rat. Data were analyzed by using one-way ANOVA followed by Tukey's post hoc test. HP: Haloperidol, FG: filgrastim.

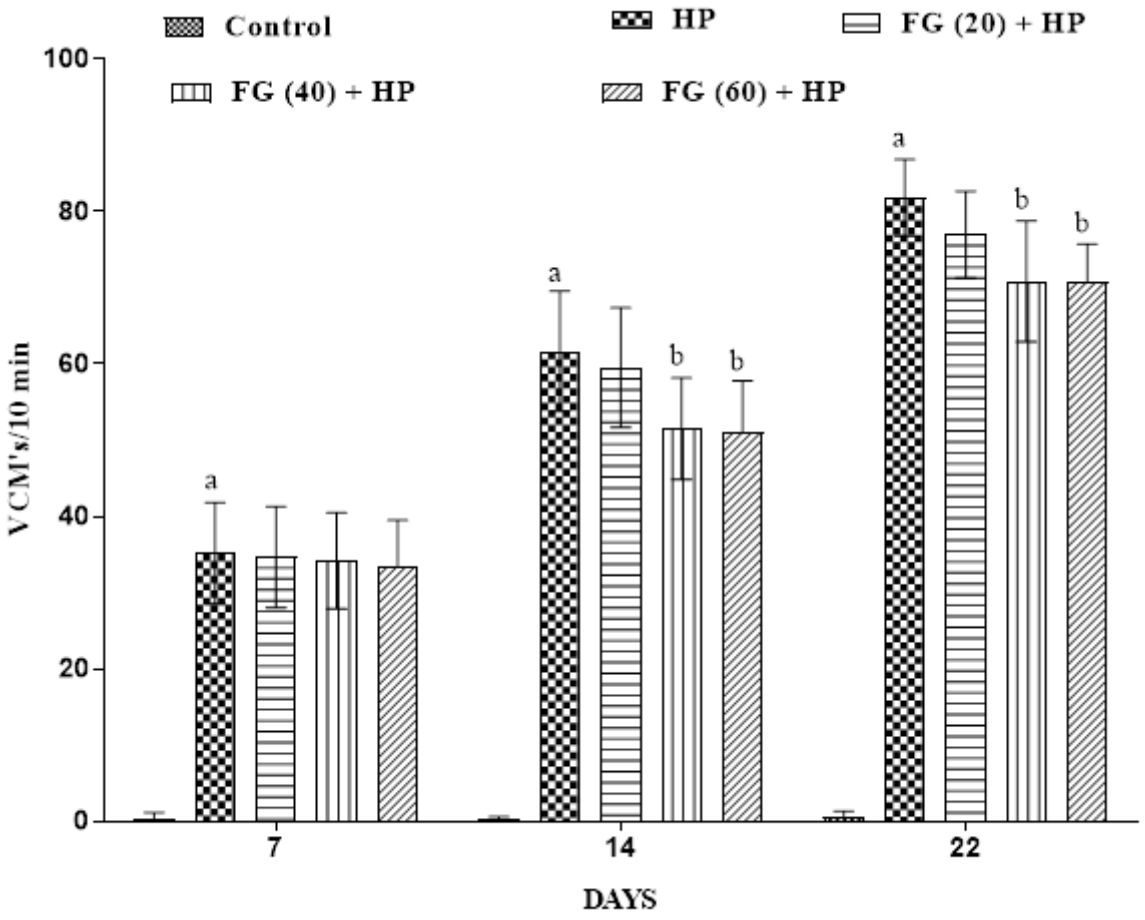

Figure 4

Effect of filgrastim on vacuous chewing movements in haloperidol treated rats. Data were analyzed by using two-way ANOVA followed by Bonferroni's post hoc test for multiple comparison. ap <0.001 Vs control, bp <0.05 Vs haloperidol. HP: Haloperidol, FG: filgrastim, VCM: vacuous chewing movements.

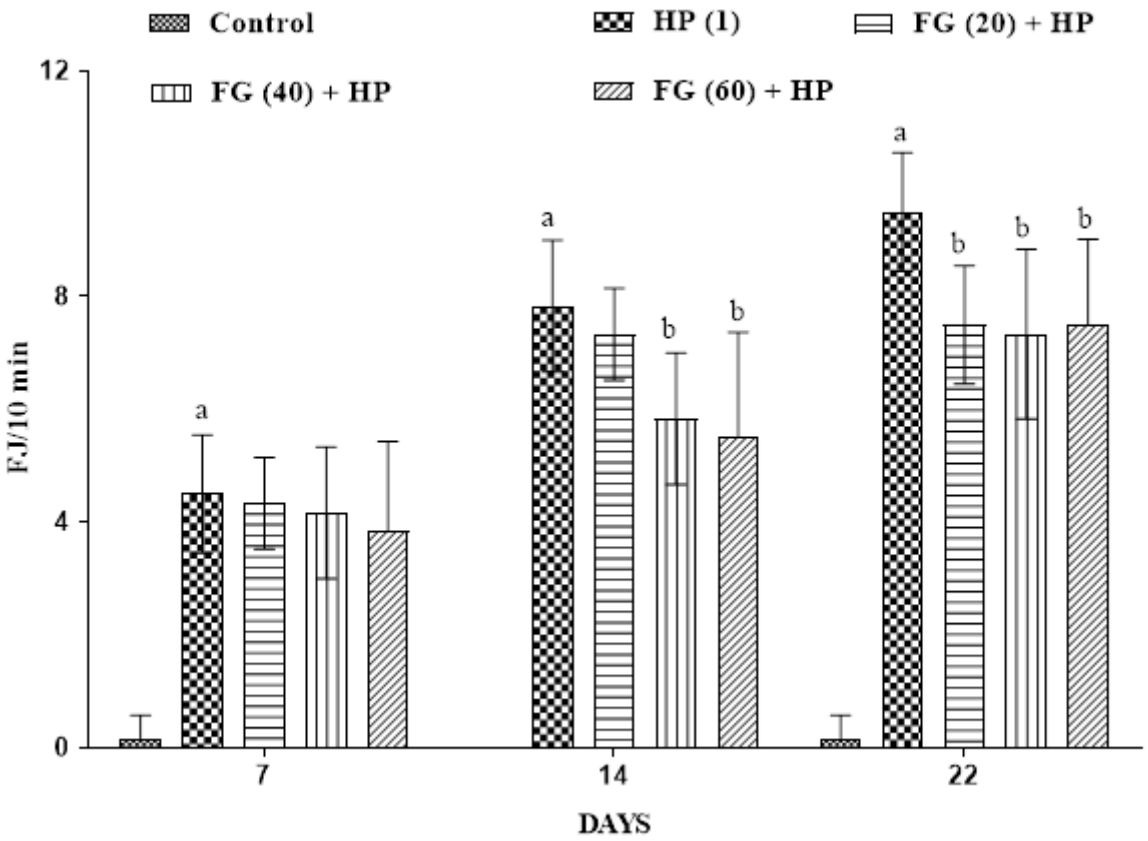

Figure 5

Effect of filgrastim on haloperidol induced facial jerking in rats. Data were analyzed by using two-way ANOVA followed by Bonferroni's post hoc test for multiple comparison. ap <0.001 Vs control, bp <0.05 Vs haloperidol. HP: Haloperidol, FG: filgrastim, FJ: facial jerking.

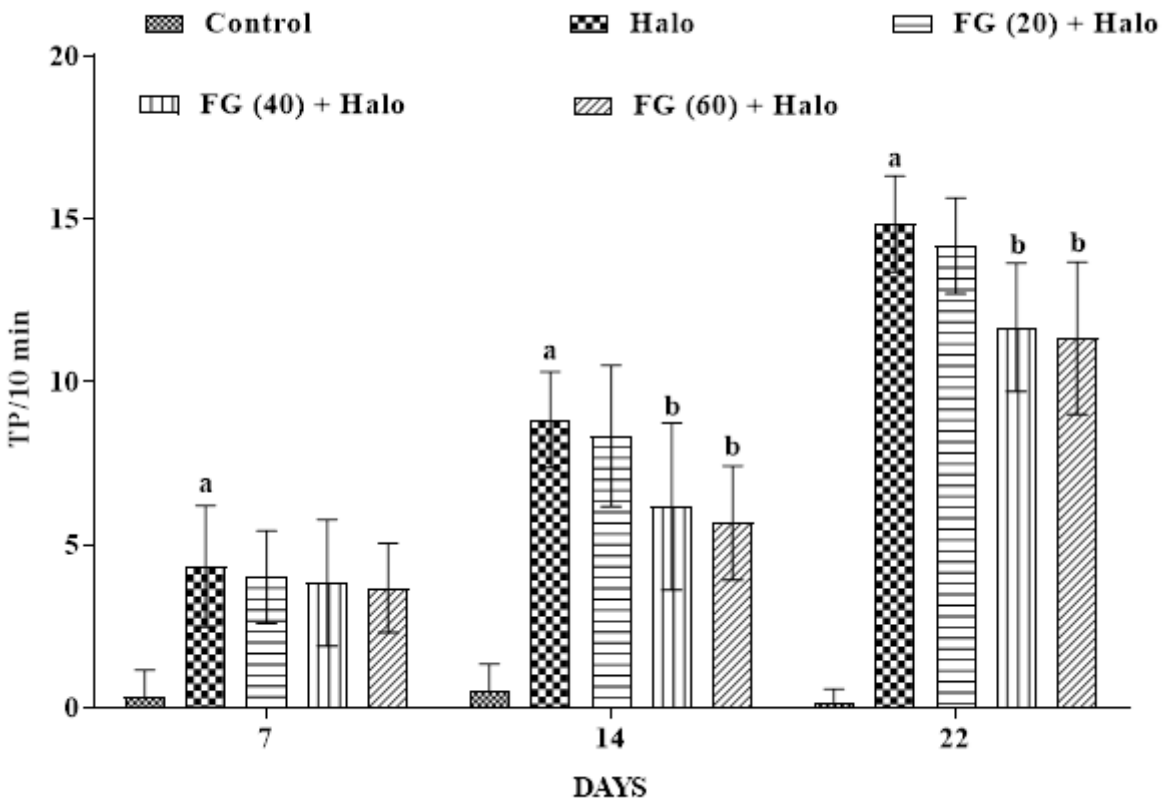

Figure 6

Effect of filgrastim on haloperidol induced tongue protrusion in rats. Data were analyzed by using two-way ANOVA followed by Bonferroni's post hoc test for multiple comparison. ap <0.001 Vs control, bp <0.05 Vs haloperidol. HP: Haloperidol, FG: filgrastim, TP: tongue protrusion.

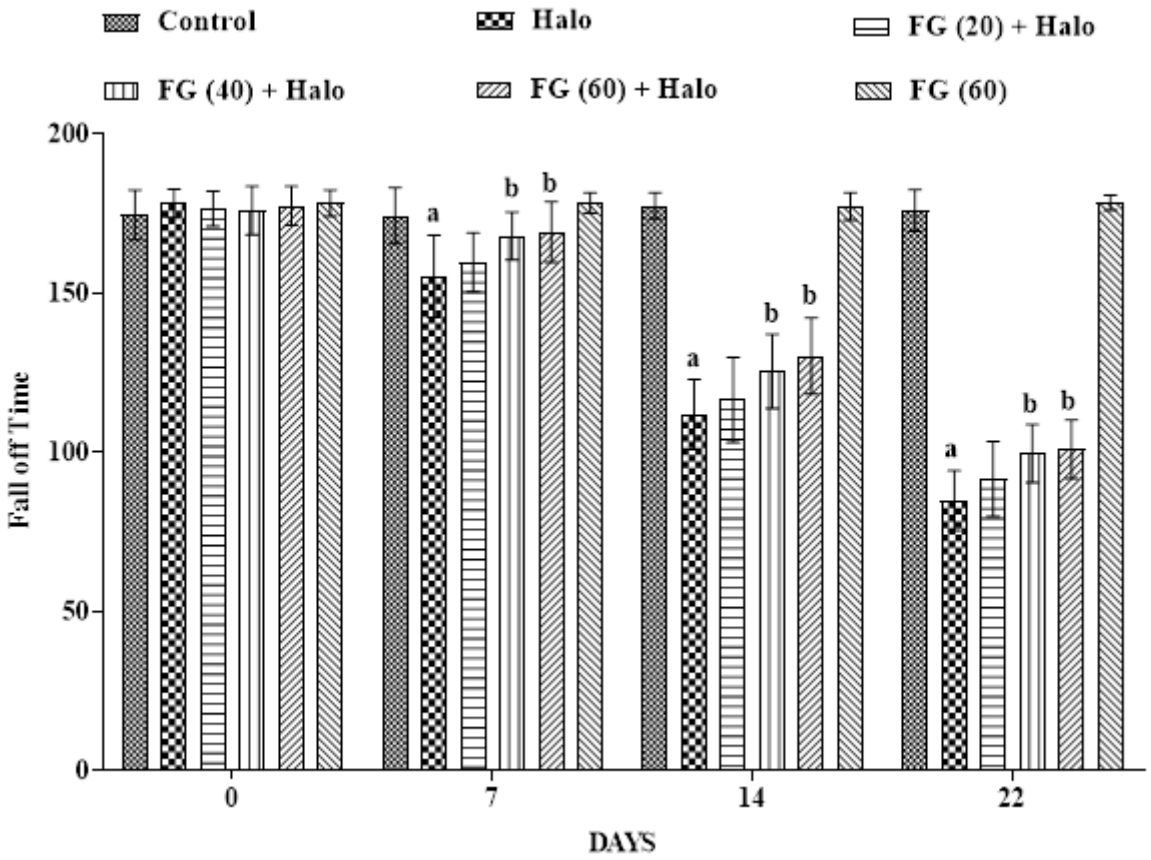

Figure 7

Effect of fillgrastim on Rotarod activity in haloperidol treated rats. Data were analyzed by using two-way ANOVA followed by Bonferroni's post hoc test for multiple comparison. ap <0.001 Vs control, bp <0.05 Vs haloperidol. HP: Haloperidol, FG: filgrastim.

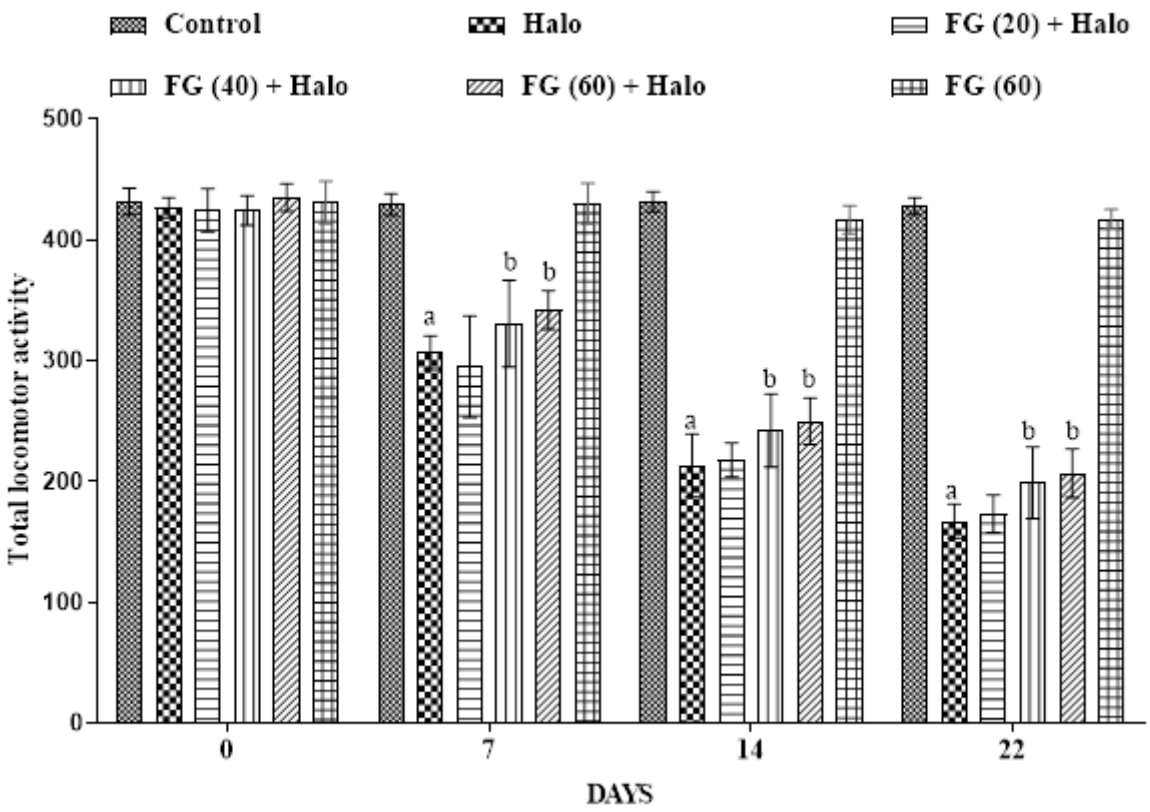

Figure 8

Effect of filgrastim on locomotor activity in haloperidol treated rats. Data were analyzed by using two-way ANOVA followed by Bonferroni's post hoc test for multiple comparison. ap <0.001 Vs control, bp <0.05 Vs haloperidol. HP: Haloperidol, FG: filgrastim.

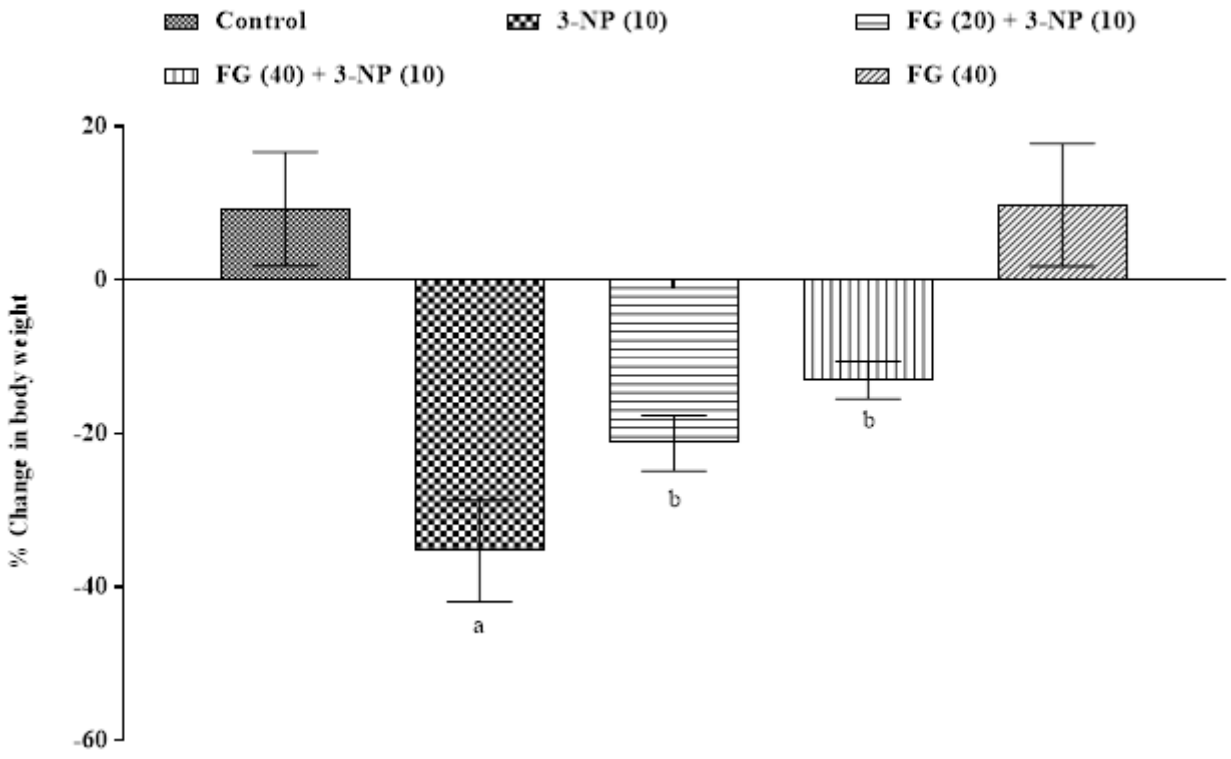

Figure 9

NP induced HD like symptoms Effect of filgrastim on body weight in 3-NP treated rats. Data analyzed by one-way ANOVA followed by Tukey's post hoc test, ap<0.001 versus control, bp<0.05 Vs 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim

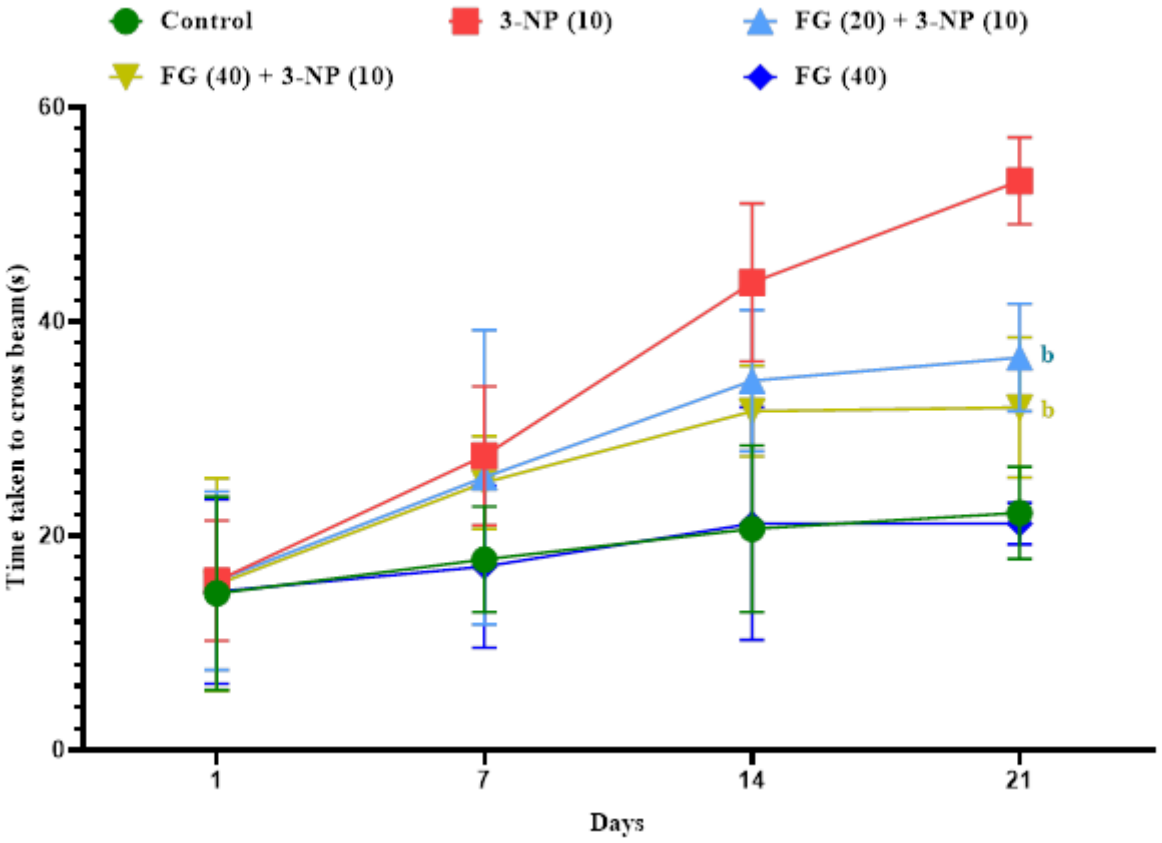

Figure 10

Effect of filgrastim on time taken to cross beam (s) in 3-NP treated rats (n = 6). Data analyzed by two-way repeated measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 Vs control, bp<0.05 Vs 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim

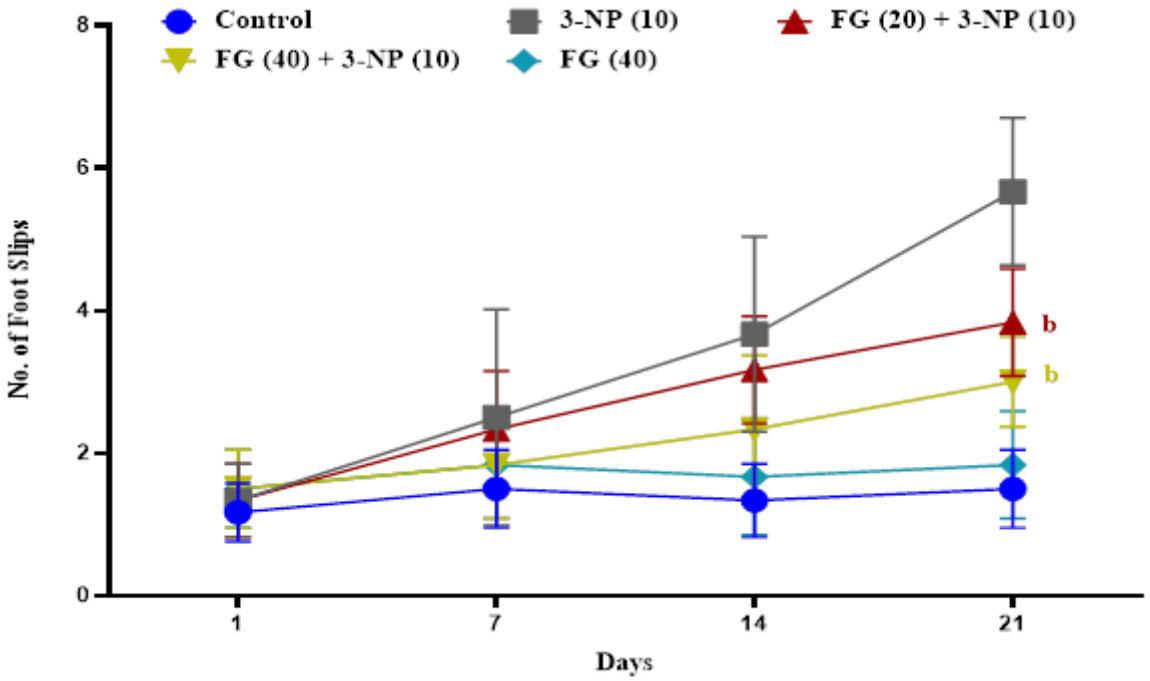

Figure 11

Effect of filgrastim on No. of foot slips in 3-NP treated rats. Mean ± S.D. Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 Vs control, bp<0.05 Vs 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim

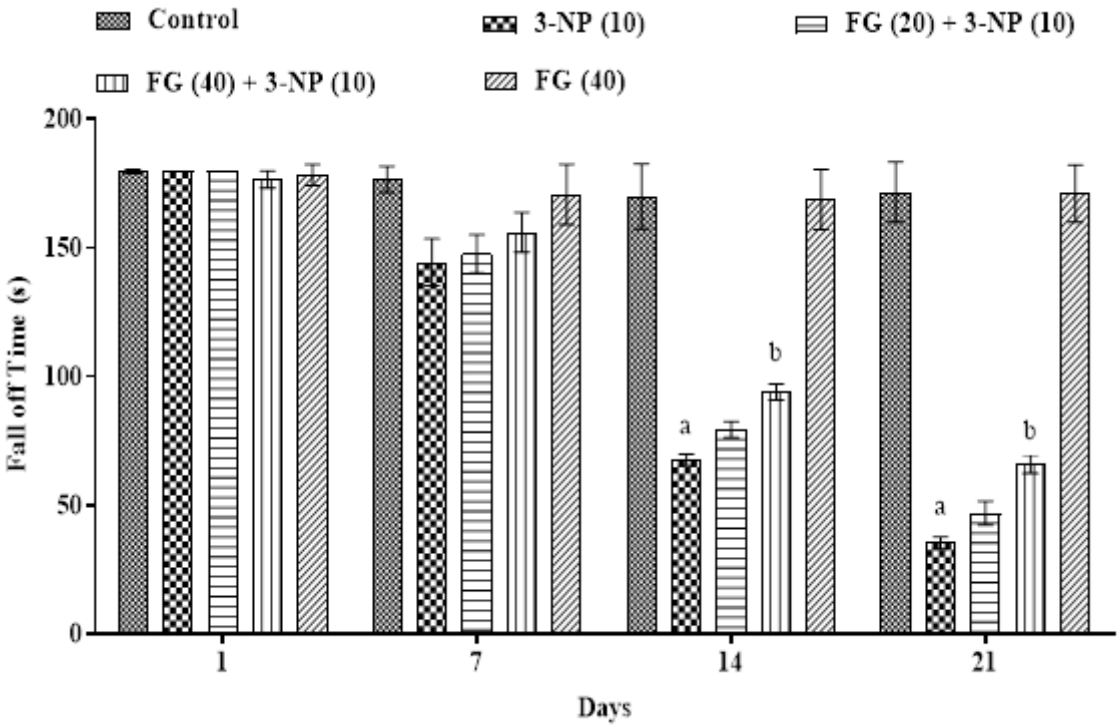

Figure 12

Effect of filgrastim on rotarod activity in 3-NP-treated rats. Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 versus control, bp<0.05 versus 3-NP, cp<0.05 versus FG 20. 3-NP=3-Nitropropinic acid, FG= Filgrastim

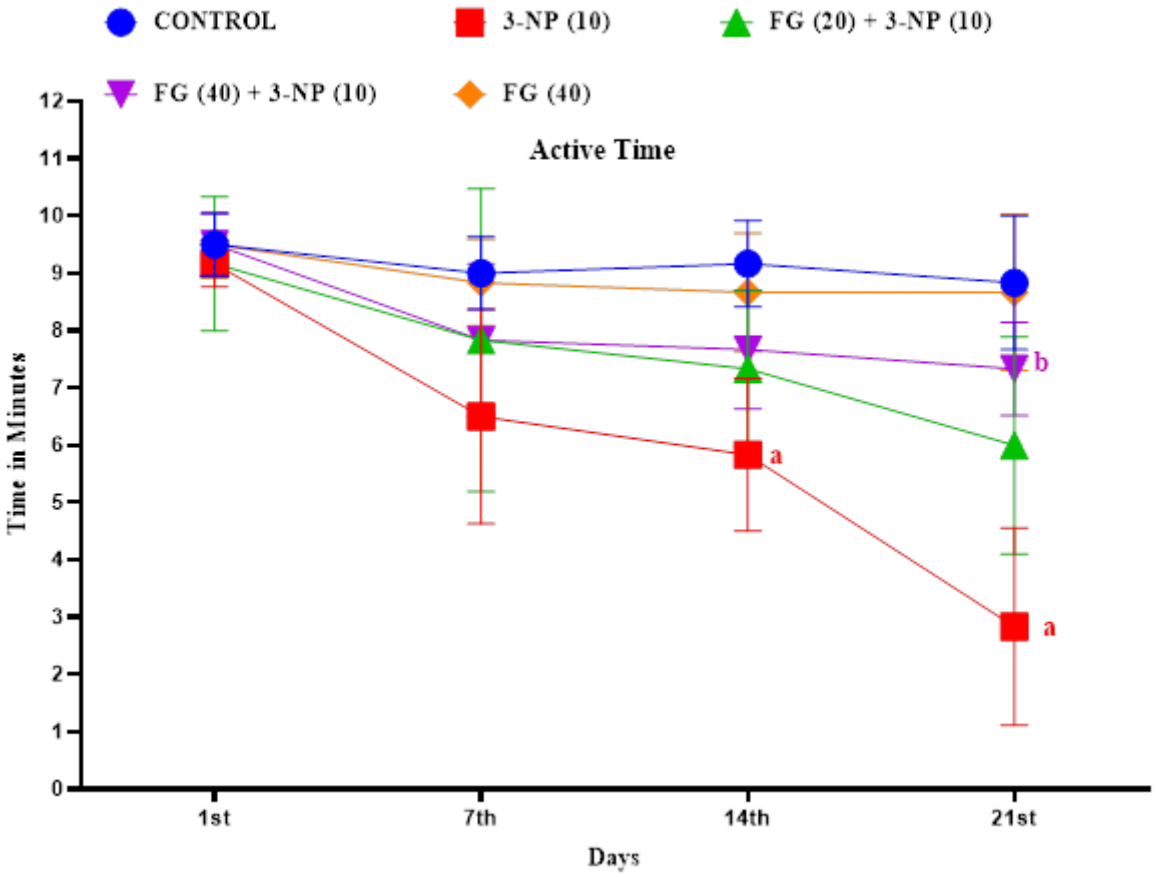

Figure 13

Effect of filgrastim on active time spent in open field apparatus in 3-NP treated rats. Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 versus control, bp<0.05 versus 3-NP, Mean ± S.D. ap<0.05 vs control, bp<0.05 vs 3-NP 3-NP=3-Nitropropinic acid, FG= Filgrastim

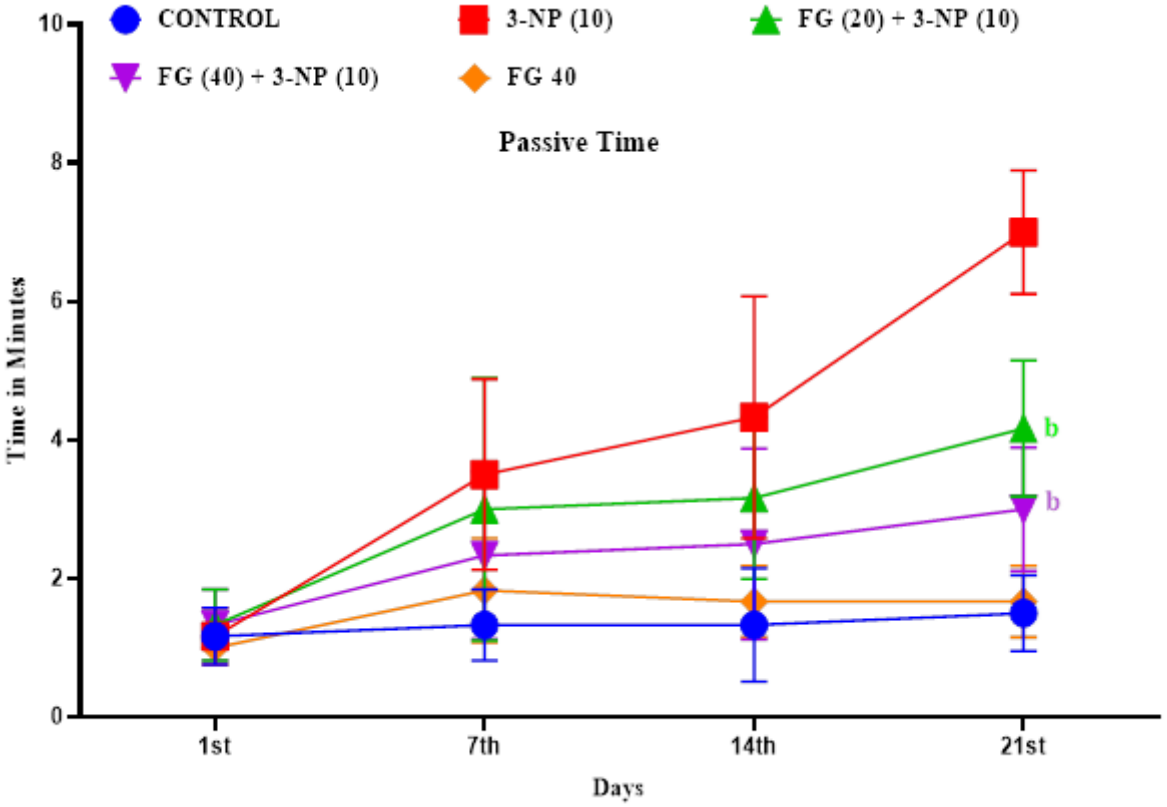

Figure 14

Effect of filgrastim on Passive time spent in open field apparatus in 3-NP treated rats (n=6). Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 versus control, bp<0.05 versus 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim

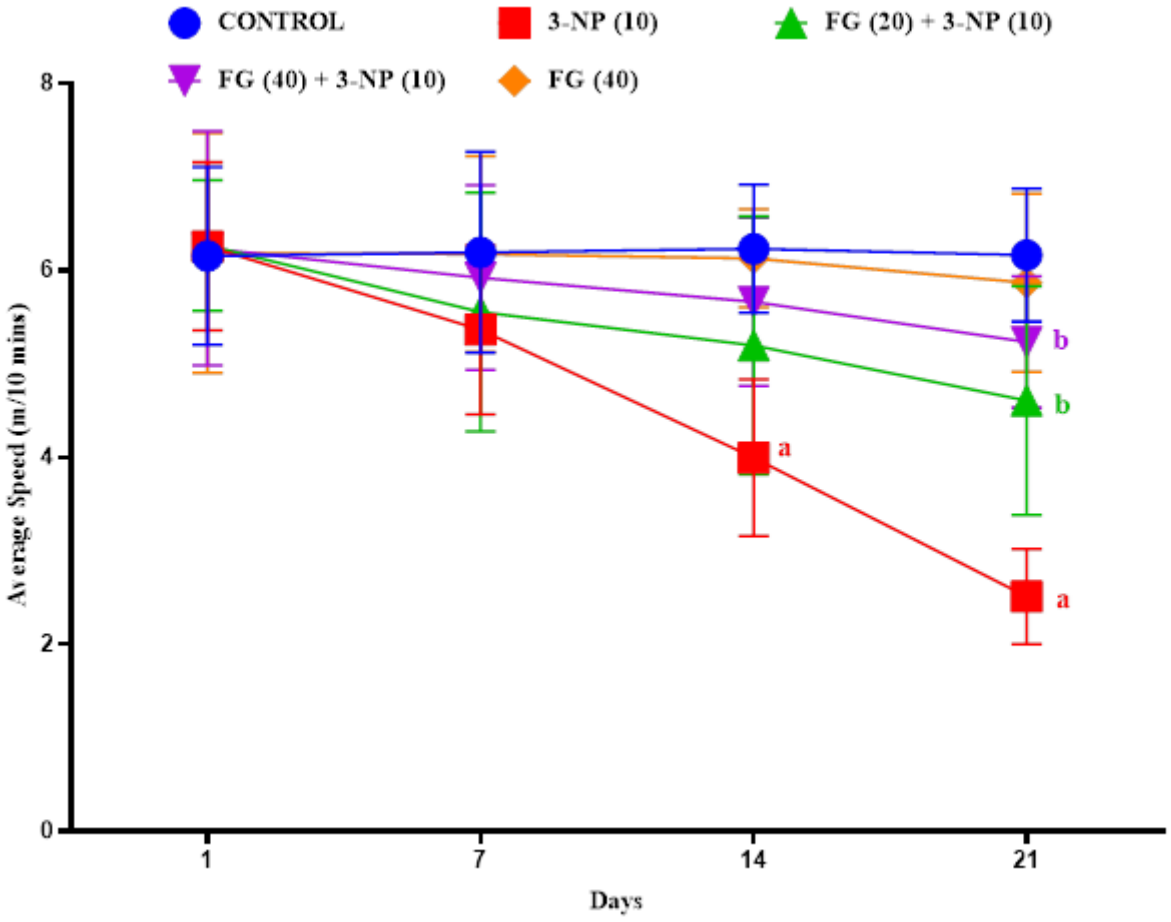

Figure 15

Effect of filgrastim on average speed in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 versus control, bp<0.05 versus 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim

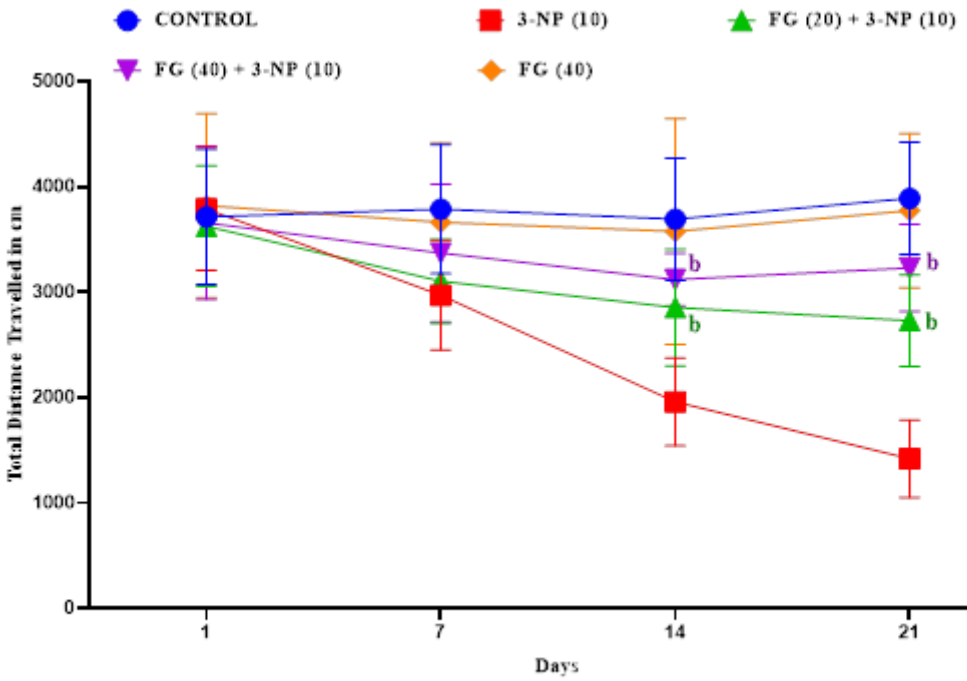

Figure 16

Effect of filgrastim on total distance travelled in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 versus control, bp<0.05 versus 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim

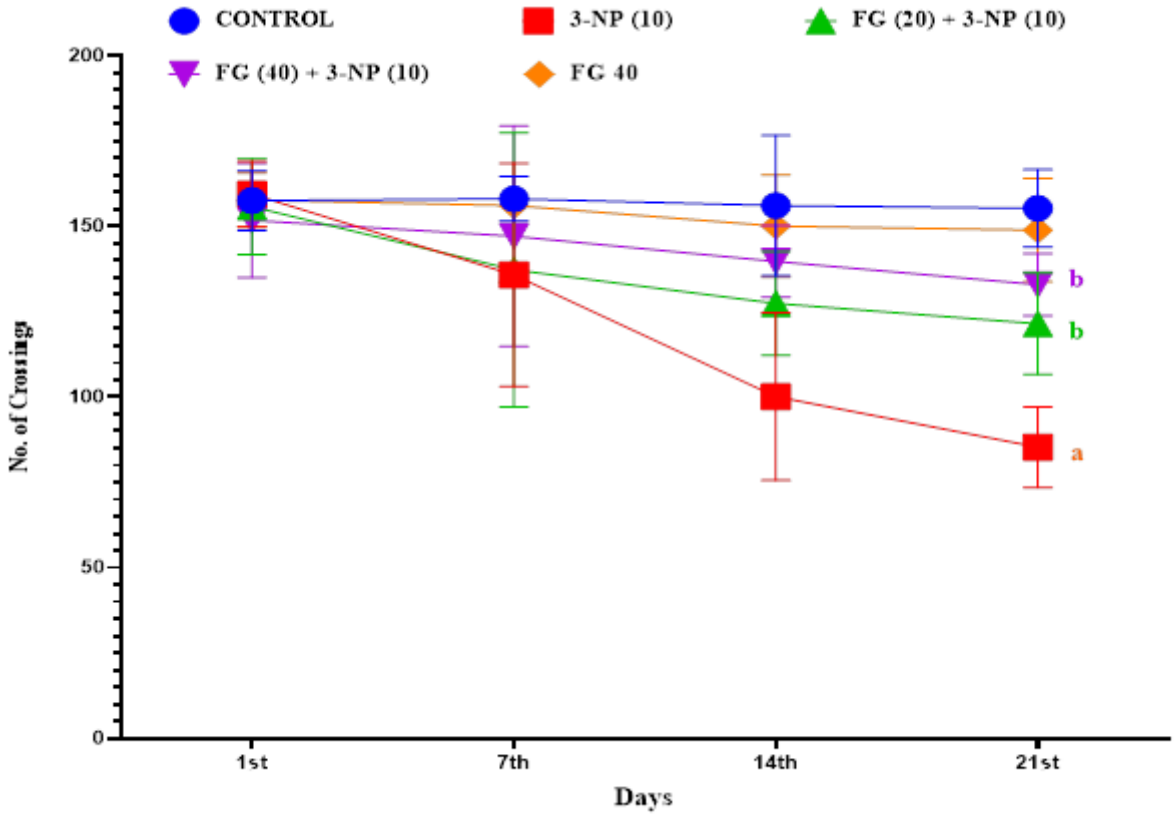

Figure 17

Effect of filgrastim on number of crossings in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated measures ANOVA followed by Bonferroni's multiple comparison. ap<0.001 versus control, bp<0.05 versus 3-NP. 3-NP=3-Nitropropinic acid, FG= Filgrastim