

Ameliorative Effect of Nebivolol in Parkinson's disease

Vandana S. Nade*, Laxman A. Kawale, Shankar S. Zambare, Pranita P. Dharmadhikari
and Priyanka S. Pagare

*Department of Pharmacology, M.V.P.S. Colleges of Pharmacy,
Gangapur Road, Nashik -422002, India*

*Corresponding author e-mail: kawalevl@rediffmail.com

ABSTRACT

Objective: The objective of the present study was to evaluate anti-parkinsons activity of nebivolol.

Methods: Parkinson's disease (PD) was induced by administration of rotenone (3 mg/kg/day, i.p for 21 consecutive days), and haloperidol (1 mg/kg, i.p). The symptoms of PD like tremors, akinesia, rigidity and catalepsy were evaluated. Foot shock-induced aggression (FSIA) model was used to confirm anti-parkinsonian activity. Nebivolol was administered at doses of 5, 10 and 20 mg/kg, p.o.

Results: Treatment with nebivolol significantly reduced intensity of muscular rigidity, akinesia, tremors, duration of catalepsy and increase fighting behaviour. The locomotor activity, exploratory behavior and grip strength were significantly improved by nebivolol. In rotenone model, the biochemical analysis of brain revealed the increased level of lipid peroxidation (LPO) and decreased levels of superoxide dismutase (SOD) and catalase (CAT). Treatment with nebivolol significantly reduced LPO level and restored the defensive antioxidant enzymes SOD and CAT.

Conclusion: Nebivolol may be used as a neuroprotective agent in the treatment of parkinsons disease along with standard anti-parkinson agents.

Keywords: Foot shock-induced aggression, Neurodegeneration, Parkinson's disease, Rotenone.

INTRODUCTION

Neurodegeneration is a condition in which cells of brain and spinal cord are lost. Neurodegenerative diseases share several common features¹. Examples of these

conditions are Parkinson's disease, Alzheimer's disease and Huntington disease. The etiology of Parkinson's disease is still unknown, although participation of

environmental toxins and oxidative stress are postulated². Neuronal dysfunction is strongly related to oxidative stress generated by formation of reactive oxygen species (ROS)³.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by severe motor deficits, often followed by cognitive dysfunction with progression of the disease. Typical onset of motor symptoms occurs after age 50, and they include tremors, akinesia, muscular rigidity, and postural instability. The imbalance between dopamine deficiency and over activity of cholinergic system gives rise to this motor disorder. The rational approach to the therapy of PD is correction of this imbalance^{4,5}. Therefore, the drug therapy in PD would be either to increase central dopaminergic activity or to decrease the central cholinergic activity.

Nebivolol is the selective β_1 adrenergic blocker with discrete pharmacological properties compared with other drugs exhibiting β blocking action. Nebivolol, along with its antihypertensive action, possesses a direct scavenging activity on oxygen radicals with peculiar antioxidant properties, which could play a key role in neurodegenerative disorders⁶.

Nebivolol possesses antidepressant, anticonvulsant, nootropics and antioxidant properties but we found focus of research on nebivolol was not on Parkinson's disease. In few literatures, it was found that propranolol and other β -adrenergic antagonist suppress the Parkinson's like symptoms such as tremor, akinesia and muscular rigidity and also Suppress of cholinergic activity in brain.⁶ Therefore, the present investigation was done to evaluate the effectiveness of nebivolol in neurodegeneration associated with Parkinson's disease.

MATERIALS AND METHODS

Animals

Male wistar rats (230-250 g) were used for the study. Animals were housed in polypropylene cages and maintained at a constant temperature of $25 \pm 2^\circ\text{C}$ with 12:12 h L/D cycle and $50 \pm 5\%$ relative humidity and were fed with standard laboratory food and water *ad libitum*. Animals were acclimatized to laboratory conditions before study. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and the Institutional Animal Ethical Committee (IAEC) of M.V.P.S College of Pharmacy, Nasik, India approved protocol of this study (IAEC/2014/03).

Drugs and chemicals

Nebivolol (Hetero Labs, Hyderabad, India), Levodopa-carbidopa (Glaxosmithkline Inc. India), Rotenone (Sigma-Aldrich, USA), Haloperidol (RPG Life science, Ankleshwar, India), Nitrobluetetrazolium chloride (NBT) (HiMedia Laboratories Pvt. Ltd. Mumbai, India) DMSO (Modern Industries, Nashik, India). All chemicals used were of analytical grade and purchased from standard manufacturers.

EXPERIMENTAL DESIGN

Rotenone-induced neurodegeneration

Rotenone, a naturally occurring lipophilic compound obtained from the roots of certain plants (Derris species) and also used as the main component of many herbicides. Acute or chronic administration of rotenone in rats reproduces PD features like muscular rigidity, tremors, akinesia and impairment in the motor functions⁷. Rotenone was used to induce Parkinson's symptoms in rats. The rats were randomly divided into six groups ($n = 5$). Group I

(control): Vehicle (PEG and DMSO as vehicle in ratio 1:1 in distilled water 5 ml/kg, i.p.), Group II: Rotenone (3 mg/kg, i.p. dissolved in PEG and DMSO as vehicle in ratio 1:1), Group III: rotenone + nebivolol (5 mg/kg p.o.), Group IV: rotenone + nebivolol (10 mg/kg p.o.), Group V: rotenone + nebivolol (20 mg/kg p.o.) and Group VI: rotenone + levodopa-carbidopa (30 mg/kg, p.o.). A time interval of 30 min was maintained in between administration of rotenone and test/standard drug. Standard and test drugs were administered for a period of 21 days⁸.

Antiparkinson's activity

On the 21st day, 24 h of the last treatment, animals were observed for the Parkinson's symptoms like muscular rigidity, akinesia and tremors. Animals were tested for muscular rigidity using horizontal rod (diameter 0.5 cm) made of glass and was kept at a height of 25 cm above the bottom surface. Each rat was suspended with forelimbs at middle part of rod. The time to fall on the bottom surface was noted. The cut-off time was kept 1 min⁹.

The severity of tremors was measured by giving scores such as no tremors (0), occasional twitches (1), moderate twitches (2) and continuous tremors (3)¹⁰.

To measure the akinesia in rats, tail of the animal was held in hand and animal was put for forward movements on his forelimbs. The number of steps taken with the forelimbs of animal was counted for three minutes¹¹.

Behavioural study

Locomotor activity

Actophotometer operates on photoelectric cells which are connected in circuit with a counter. When the beam of

light falling on the photocell is cut off by the animal, a count was recorded for 10 min¹².

Exploratory behavior (Hole board test)

The hole-board has a size of 40 × 40 cm and 16 holes with a diameter of 3 cm each are distributed evenly on the floor. The animals were allowed to explore the holes in the board for 5 min, and the total number of pokes per 5 min was measured by visual observation for each rat¹³.

Grip strength

The grip strength was measured by using grip strength meter (Orchid, India). The apparatus consisted of an adjustable trough and a push-pull strain gauge with a triangular brass ring which was grasped by the animal with its forelimbs. The animal was pulled on the tail until the grip was loosened. The animal continues to be pulled along the trough until the hind limbs grasp a T-shaped bar being also attached to push-pull strain gauge. The trial was completed when the grip of the hind limbs was also loosened. Fore and hind limb strength were measured. The reading of the grip strength was recorded by grip strength meter by using the software¹⁴.

Biochemical estimation

Preparation of tissue homogenate

Brains of rats were isolated. Brain was immediately washed in ice-cold saline and weighed. A 10% (w/v) tissue homogenate was prepared in ice-cold 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for catalase assay was obtained by centrifugation (Remi – C, – 30 Remi Industries Ltd. Mumbai, India) of the homogenate at 1000 rpm for 20 min, at 4⁰ C; for other enzyme assays, centrifugation was at 12,000 rpm for 60 min at 4⁰ C. Bio-Spectrophotometer (Elico, B-200) was used for subsequent assay¹⁵.

Catalase Activity

Catalase (CAT) activity was assayed by the method of Luck, 1971; wherein the breakdown of H_2O_2 is measured at 240 nm. Briefly, the assay mixture consist of 3 ml of H_2O_2 - phosphate buffer (pH 7) and 0.05 ml of supernatant of tissue homogenate (10%), the change in absorbance was recorded after 1 min at 240 nm. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.071). The results were expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein¹⁶.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed according to the method of Kono, 1978 wherein the reduction of nitrobluetetrazolium (NBT) was inhibited by the SOD and was measured spectrophotometrically at 560 nm. Briefly, the reaction was initiated by the addition of the hydroxylamine hydrochloride to the reaction mixture containing NBT and the post nuclear fraction of the homogenate (10%). The result was expressed as unit per milligram of protein, with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50 %¹⁷.

Lipid peroxidation assay

The quantitative measurement of lipid peroxidation (LPO) in rat brain was done by the method of Wills, 1966. The reaction was initiated by addition of 0.2 ml of 8% SLS, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA) to 0.1 ml of tissue homogenate. Finally, the volume was made to 4.0 ml by adding distilled water and then heated at 95°C for 1 h on water bath and cooled to room temperature. Then, 5 ml mixture of n-butanol: Pyridine (15:1 by

volume) was added to it and shaken vigorously for 10 minutes. The amount of malondialdehyde (MDA) formed was measured by reaction with thiobarbituric acid at 532 nm. The results were expressed as nanomoles of MDA per milligram of protein, using the molar extension coefficient of chromophore ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$)¹⁸.

Haloperidol-induced catalepsy

Haloperidol, an antipsychotic drug which blocks central dopamine receptor in striatum, Produces a behavioural state in animals like mice and rats in which they fail to correct externally imposed postures. This is referred as catalepsy^{19, 20}. The rats were randomly divided into six groups (n = 5). Animals were given treatment in different groups as follows: Group I (control): Vehicle (Distilled water, 5 ml/kg, i.p.), Group II: Haloperidol (1 mg/kg, i.p.), Group III: Haloperidol + nebivolol (5 mg/kg p.o.), Group IV: haloperidol + nebivolol (10 mg/kg p.o.), Group V: Haloperidol + nebivolol (20 mg/kg p.o.) and Group VI: Haloperidol + levodopa-carbidopa (30 mg/kg, p.o.). A time interval of 30 min was maintained in between administration of haloperidol and test and standard drugs²¹.

Foot-shock induced aggression (FSIA)

In the present investigation, we attempted to evaluate the relation between dopamine and symptoms of Parkinsonism²². The animals were divided into six groups, each group consisted of five pairs (n = 10) such that fighting behaviour can observed into the chamber of foot-shock apparatus. Animals were given treatment in different groups as follows: Group I (Control): Vehicle (5 ml/kg, i.p.), Group II: Foot-shock (60 Hz, 5 s followed by 5 s for 3 min.), Group III: Foot-shock + nebivolol (5 mg/kg p.o.), Group IV: Foot-shock + nebivolol (10 mg/kg p.o.), Group V: Foot-shock +

neбиволл (20 mg/kg p.o.) and Group VI: Foot-shock + леводопа-карбидопа (30 mg/kg p.o.). The two rats were placed in a box with grid floor consisting of rods with a distance of 5 mm. A constant current of 0.8 mA was supplied to the grid floor by a LVE constant current shocker. A 60-Hz current was delivered for 5 s followed by 5 s intermission for 3 min. Each pair of rat was dosed and tested without previous exposure. The total numbers of fights were recorded for each pair during the 3 min period²³.

Statistical Analysis

Results were expressed as mean $\pm$ SEM, and the statistical analysis of data was done using one-way analysis of variance (ANOVA).

RESULTS

Rotenone-induced neurodegeneration

Muscular rigidity

The intensity of muscular rigidity was significantly increased in rotenone treated group as compared to vehicle group. Treatment with небиволл (10 and 20 mg/kg) significantly decreased muscular rigidity ($p < 0.05$, $p < 0.01$ respectively) as compared to rotenone treated group. The muscular rigidity was also significantly decreased in леводопа-карбидопа treated group ($p < 0.001$) as compared to rotenone treated group (Table 1).

Tremors

Animals treated with rotenone showed significant ($p < 0.001$) increase in the number of tremors as compared to vehicle treated group. Administration of небиволл (20 mg/kg) significantly decreased ($P < 0.05$) the number of tremors as compared to rotenone treated group. Tremors were also significantly decreased in леводопа-карбидопа treated group ($p < 0.01$) (Table 1).
Akinesia

The rotenone administered animals showed significantly ($p < 0.001$) increase in akinesia as compared to vehicle treated group. Akinetic behaviour of animals was significantly decreased in небиволл (5, 10 and 20 mg/kg) ($p < 0.01$, $p < 0.001$, $p < 0.001$ respectively) as compared to rotenone treated group. The akinesia was also decreased in леводопа-карбидопа treated group ($p < 0.001$) (Table 1).

Locomotor activity

Total locomotor activity of rats in rotenone treated group was significantly ($p < 0.001$) reduced as compared to vehicle group. Administration of небиволл (10 and 20 mg/kg) showed significant ($p < 0.001$) increase in the locomotor activity as compared to rotenone treated group. Леводопа-карбидопа significantly ($p < 0.001$) increased locomotor activity (Table 2).

Exploratory behaviour

Animals treated with rotenone showed significant ($p < 0.001$) reduction in number of nose pokes as compared to vehicle treated group. Treatment with небиволл (5, 10 and 20 mg/kg) significantly ($p < 0.05$, $p < 0.001$, $p < 0.001$ respectively) increased number of pokes as compared to rotenone treated group. Number of pokes was also increased in леводопа-карбидопа treated group ($p < 0.001$) (Table 2).

Grip strength

Rotenone treated animals showed significant ($p < 0.001$) reduction of grip strength as compared to vehicle treated group. Treatment with небиволл (10 and 20 mg/kg) and леводопа-карбидопа treated group showed significant ($p < 0.001$, $p < 0.001$) increase of grip strength as compared to rotenone treated group when measured by grip strength meter (Table 2).

Biochemical effects

Effect on CAT and SOD

Tissue homogenate of brain of vehicle-treated group showed normal levels of CAT and SOD. Rotenone-treated group showed significant ($p<0.001$) decrease in SOD and CAT levels as compared to vehicle treated group, indicating induction of neurodegeneration. Treatment with neбиволol and combination of L-dopa and carbidopa showed significant ($p<0.01$) increase in SOD and CAT levels compared to rats treated with rotenone treated group (Table 3).

Effect on LPO levels

MDA levels were significantly ($p<0.001$) higher in rats treated with rotenone as compared to vehicle group; while treatment with neбиволol and combination of L-dopa and carbidopa significantly ($p<0.01$) lowered LPO levels when compared to rotenone-treated rats (Table 3).

Haloperidol-induced catalepsy

The duration of catalepsy was significantly increased ($p<0.001$) after treatment with haloperidol as compared to vehicle treated group. Treatment with neбиволol (10 and 20 mg/kg) significantly ($p<0.001$) reduced duration of catalepsy as compared to haloperidol treated group. Duration of catalepsy was also decreased in levodopa-carbidopa ($p<0.001$) treated group as compared to haloperidol treated group (Table 4).

Foot-shock induced aggression

The number of fights was significantly increased in neбиволol (10 and 20 mg/kg) ($p<0.001$, $p<0.001$ respectively) treated group as compared to foot-shock treated group. The number of fights was also significantly increased in levodopa and carbidopa ($p<0.001$) treated groups as

compared to foot-shock treated group (Figure 1).

DISCUSSIONS

The present study demonstrated anti-parkinson's activity of neбиволol in different animal models. The symptoms of Parkinson's disease were induced by administration of rotenone and haloperidol in experimental animals^{8,21}. The foot-shock induced aggression model was used to further clarify anti-parkinson's activity of neбиволol²³. Rotenone induced muscular rigidity; tremors and akinesia were considered as parameters to evaluate anti-parkinson's activity of neбиволol²⁴. Neбиволol (5, 10 and 20 mg/kg, p.o.) showed significant reduction in these symptoms in rats. Neбиволol was also able to increase the locomotor activity, exploratory behaviour and grip strength in rats. Amelioration of symptoms of rotenone by neбиволol demonstrates anti-parkinsons activity.

Oxidative stress is one of the major reasons of nerve damage in many neurodegenerative disorders. In Parkinsons disease, oxidation of dopamine by MAO-B and aldehyde dehydrogenase generates hydroxyl free radicals (OH) in presence of ferrous ions (basal ganglia are rich in iron)²⁵. So to find out oxidative stress at various levels the defensive antioxidant enzymes in rat brain were measured. SOD is the most important enzymes in the antioxidant defense system of the body. The major function of superoxide dismutase (SOD) is to catalyze the conversion of superoxide anion radicals to H_2O_2 and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions. Catalase (CAT), which is present in all mammalian cells, is responsible for the removal of H_2O_2 . LPO is the measure of the excessive oxidation of the lipids in the body indicating increased superoxide production. Therefore the oxidative stress indices were

estimated in rat brain like SOD, CAT and LPO. There was significant reduction in antioxidant defensive enzymes SOD and CAT and increase in LPO content observed in rotenone induced neurodegeneration model. The levels of SOD, CAT and LPO were significantly restored by treatment with nebivolol.

In the present study, animals were treated with haloperidol (1 mg/kg) showed cataleptic behaviour similar to the symptoms of parkinson's disease. Haloperidol-induced catalepsy is one of the animal models to test the extrapyramidal side effects of antipsychotic drugs. The haloperidol, (a non-selective D₂ dopamine antagonist) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum. The agent, increasing dopamine transmission inhibits neuroleptic induced catalepsy²⁶. The nebivolol (10 and 20 mg/kg, p.o.) showed significant reduction in duration of catalepsy demonstrating anti-parkinson activity. The inhibition of catalepsy is indicative of the ability of the drug to potentiate dopaminergic transmission in striatum.

In the present investigation, attempt has been made to study the effect of nebivolol on dopaminergic transmission. The foot-shock induced aggression (FSIA) model was used to evaluate anti-Parkinson activity of nebivolol. Aggressive behaviour is due to central monoaminergic neurons. The brain D₂ dopamine receptors play important role in the modulation of foot-shock aggressive behaviour in mice. In foot shock-induced aggression, it is found that the brain dopamine levels are increased.²³. 0.8 mA current was given to the animals. Pretreatment with nebivolol (10 and 20 mg/kg, p.o.) significantly increased the number of fighting attacks, therefore suggesting a possible dopaminergic activity of nebivolol in FSIA. Fighting behaviour

also significantly increased in animals treated with L-dopa and carbidopa.

The above behavioral and biochemical results suggest that nebivolol has the ability to improve symptoms of Parkinsonism. The anti-parkinsonism activity may be due to restoration of level of dopamine and antioxidant property of nebivolol.

CONCLUSION

Our investigation indicates that Nebivolol, selective β_1 adrenergic blocker significantly ameliorated the symptoms of parkinsons diseases. Hence, nebivolol may be useful as a neuroprotective agent in the treatment of Parkinson's disease.

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Table 1: Effect of nebivolol on muscular rigidity, akinesia and tremors in rotenone induced neurodegeneration.

Treatment (Groups)	Muscular rigidity Duration of Suspension/1 min (S)	Akinesia Number of steps taken by the animal/3 min	Tremors
I	33.99±1.8	62.6±2.06	0
II	10.21±0.65 ^{##}	21.4±1.50 ^{##}	2.4±0.24 ^{##}
III	11.87±0.98 ^{ns}	33.2±1.85 ^{**}	2.2±0.37 ^{ns}
IV	23±1.40 [*]	42.8±2.15 ^{***}	1.4±0.50 ^{ns}
V	28.01±0.79 ^{**}	50.2±3.42 ^{***}	0.8±0.37 [*]
VI	43.06±7.03 ^{***}	57.2±2.57 ^{***}	0.6±0.40 ^{**}

All values are expressed as mean ± S.E.M. (n = 5).

^{ns}Non-significant, ^{##}, ^{***} p<0.001(One-way ANOVA followed by Dunnett's test), * Group III, IV, V and VI compared to Group II. # Group II compared to Group I.

Table 2: Effect of nebivolol on locomotor activity, exploratory behavior and grip strength in rotenone induced neurodegeneration.

Treatment (Groups)	Locomotor activity (Number of locomotion/ 10 min)	Exploratory behavior (Number of nose poking/5 min)	Grip strength (N)
I	1018 ± 10.95	20.8 ± 0.583	13.61 ± 0.152
II	619.8 ± 6.69 ^{##}	8.6 ± 0.50 ^{##}	6.28 ± 0.105 ^{##}
III	624.6 ± 9.13 ^{**}	10.4 ± 0.50 [*]	6.31 ± 0.13 ^{ns}
IV	694 ± 4.01 ^{***}	11.8 ± 0.37 ^{***}	8.06 ± 0.16 ^{***}
V	789.8 ± 9.13 ^{***}	15 ± 0.31 ^{***}	10.54 ± 0.26 ^{***}
VI	902 ± 6.58 ^{***}	16.4 ± 0.50 ^{***}	11.02 ± 0.20 ^{***}

All values are expressed as mean ± S.E.M. (n = 5).

^{ns}Non-significant, ^{##}, ^{***} p<0.001(One-way ANOVA followed by Dunnett's test), *Group III, IV, V and VI compared to Group II. # Group II compared to Group I.

Table 3: Effect of nebivolol on brain SOD, CAT and LPO levels in rotenone induced neurodegeneration.

Treatment (Groups)	CAT (μ Mole of H ₂ O ₂ decomposed/mg protein/min)	SOD (% inhibition of reduction of NBT)	LPO (nMole of MDA/mg protein)
I	10.88 $\pm$ 0.421	82.58 $\pm$ 1.02	10.59 $\pm$ 0.18
II	6.39 $\pm$ 0.168 ^{##}	44.74 $\pm$ 0.835 ^{##}	21.16 $\pm$ 0.35 ^{##}
III	6.29 $\pm$ 0.18 ^{ns}	46.27 $\pm$ 1.35 ^{ns}	18.96 $\pm$ 0.60 ^{ns}
IV	7.28 $\pm$ 0.25 ^{ns}	53 $\pm$ 0.84 ^{***}	16.23 $\pm$ 0.45 ^{***}
V	8.82 $\pm$ 0.17 ^{***}	57.3 $\pm$ 0.62 ^{***}	14.02 $\pm$ 0.367 ^{***}
VI	9.99 $\pm$ 0.131 ^{***}	63.29 $\pm$ 1.30 ^{***}	12.47 $\pm$ 0.15 ^{***}

All values are expressed as mean $\pm$ S.E.M. (n = 5).

nsNon-significant, ##,*** p<0.001(One-way ANOVA followed by Dunnett's test), *Group III, IV, V and VI compared to Group II. # Group II compared to Group I.

Table 4: Effect of nebivolol on duration of haloperidol-induced catalepsy.

Treatment (Groups)	Duration of catalepsy in S				
	0 (min)	30 (min)	60 (min)	90 (min)	120 (min)
I	1.03 $\pm$ 0.08	0.99 $\pm$ 0.07	1.07 $\pm$ 0.04	1.44 $\pm$ 0.12	1.46 $\pm$ 0.16
II	1.61 $\pm$ 0.20 ^{ns}	130.4 $\pm$ 3.35 ^{###}	116 $\pm$ 4.09 ^{###}	95 $\pm$ 2.27 ^{###}	79.4 $\pm$ 4.6 ^{###}
III	1.0 $\pm$ 0.33 ^{ns}	101 $\pm$ 2.25 ^{***}	94 $\pm$ 0.6 ^{***}	71 $\pm$ 0.8 ^{***}	70 $\pm$ 0.7 [*]
IV	1.16 $\pm$ 0.10 ^{ns}	81.2 $\pm$ 4.0 ^{***}	63.8 $\pm$ 3.81 ^{***}	50.6 $\pm$ 2.6 ^{***}	39.6 $\pm$ 2.69 ^{***}
V	1.3 $\pm$ 0.16 ^{ns}	26.73 $\pm$ 2.98 ^{***}	24.39 $\pm$ 3.16 ^{***}	16.28 $\pm$ 1.46 ^{***}	15.23 $\pm$ 0.89 ^{***}
VI	1.07 $\pm$ 0.05 ^{ns}	4.09 $\pm$ 0.89 ^{***}	13.12 $\pm$ 0.39 ^{***}	7.81 $\pm$ 0.04 ^{***}	16.8 $\pm$ 0.31 ^{***}

All values are expressed as mean $\pm$ S.E.M. (n = 5).

nsNon-significant, ###,*** p<0.001(One-way ANOVA followed by Dunnett's test), *Group III, IV, V and VI compared to Group II. # Group II compared to Group I.

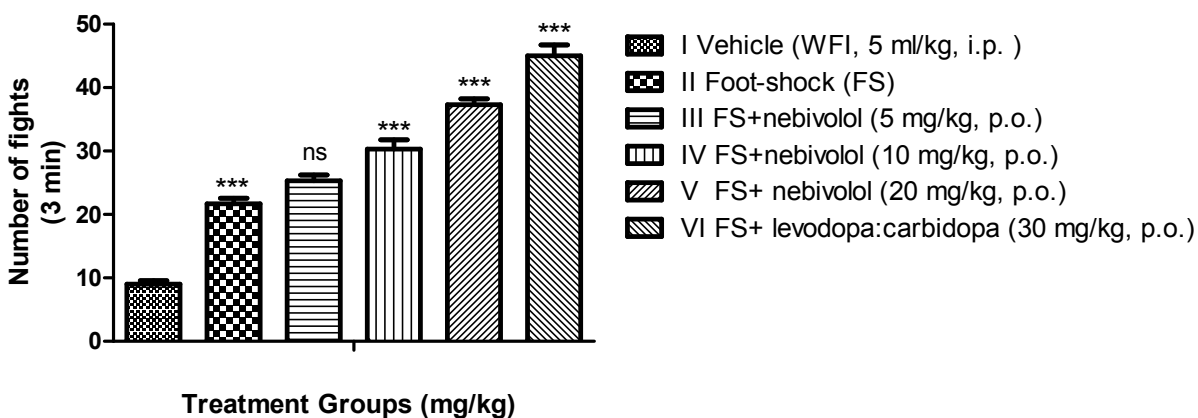


Figure No. 1: Effect of nebulol on number of fights in FSIA model.

Each column represents mean $\pm$ S.E.M. (n = 5).

Group II compared with Group I and Group III, IV, V and VI are compared with group II.

*P<0.05, **P<0.01, ***P<P.001, ns-non significant (One way ANOVA followed by Dunnett's test)