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NEUROPROTECTIVE EFFECT OF VALSARTAN (ANGIOTENSIN RECEPTOR BLOCKER) IN HALOPERIDOL INDUCED PRECLINICAL ANIMAL MODEL OF PARKINSON'S DISEASE

Jegan Nallamadan*[1], N. Chidambaranathan[1], G. Nalini[1], K. Marikrishnaa[1]

[1*]Department of Pharmacology, K. M. College of Pharmacy, Madurai, Tamilnadu, India.

ABSTRACT

Parkinson's disease is a progressive neurodegenerative disorder characterised by the selective loss of dopaminergic neuron of substantia nigra pars compacta. Haloperidol (intraperitoneally in the dose of 2mg/kg) is commonly used to induce Parkinson‟s disease in rat. It blocks dopamine receptors present in the straitum, thus induces Parkinson‟s disease. Valsarten (ARBs) are widely used compounds therapeutically effective in cardiovascular disorders, renal disease, the metabolic syndrome, and diabetes. It has been more recently recognized that are neuroprotective and have potential therapeutic use in many brain disorders. Aim of this study to compare Valsarten with standard drug *in vivo* neuroprotective in haloperidol induced animal model in Parkinson's disease. Animals treated with Valsarten (Group IV) showed a nonsignificant difference in the 0th day (185.35) and then a gradually increase in locomotor activity on the 4th and 7th day respectively (165.23 ± 21.8 and 178.58), on the 14th day it further increase to (198.34). The increased motor rigidity was same in all the groups on 7th day one day after stopping the Parkinson inducing drug haloperidol. No significant difference was observed between the treated and untreated groups on 7th day. On 14th day much more recovery of muscle tone was noted in all the groups evidenced by rota rod. But complete recovery was not observed in all the experimental groups 7 days after stopping the standard and investigational drugs. Valsarten treated group (Group IV), a significant decrease in catalepsy was seen on 4th and 7th day as compared to the Haloperidol treated group (Group II) and increase on 14th day of the treatment. Whereas no significant difference in catalepsy was seen when Group VI compared to standard treated group (Group III). It confirmed the neuroprotective effect Valsartan in Haloperidol induced Parkinson's Disease.

KEYWORDS

Parkinson's disease, Valsartan and Haloperidol.

Author for Correspondence:
Jegan Nallamadan,
Department of Pharmacology,
K. M. College of Pharmacy,
Madurai, Tamilnadu, India.

Email: jeganrajam79@gmail.com

INTRODUCTION

Parkinson's is the second most common neurodegenerative disease affecting 1% of population including both men and women equally above 55yrs of age. It can be of unknown origin as primary or secondary due to some drugs/ stroke/trauma or purely genetical when it is manifested as early onset before 55yrs.

Loss of 50-70% of dopaminergic neurons in substantia nigra compacta (SNc) and in striatum associated with the presence of intra-cytoplasmic Lewy bodies due to aggregation of α-synuclein and ubiquitin are the basic pathological features[1]. Four cardinal clinical signs of Parkinson are tremor, rigidity, Akinesia/hypokinesia and postural instability. Other parts of CNS like dorsal motor neuron of Vagus, Nucleus basalis of Mayner, locus ceruleus and Hypothalamus are also affected even extending outside CNS like myentric plexus evidenced by the presence of Lewy bodies. These features account for non motor symptoms like sleep disturbances, depression, cognitive impairment, anosmia, constipation, incontinence and ANS dysfunctions[2]. Death of dopamine neurons has been linked to mitochondrial dysfunction, oxidative stress, nerve inflammation and insufficient autophasic proteosomal degeneration. Mutation of genes LLRK, PARK-2 (encode parkin), PARK-7, PINK-1 and SNC-A (coding α-synuclein) have been attributed only to 10% of cases. Environmental exposure to organ chlorine pesticides, polychlorinated phenols and herbicides like Paraquat also have been shown to be associated with increase in risk and occurrence of Parkinson's due to their oxidative stress and neurotoxic effects by many epidemiological studies[3]. Angiotensin II receptor blockers (ARBs, collectively called sartans) are widely used compounds therapeutically effective in cardiovascular disorders, renal disease, the metabolic syndrome, and diabetes. It has been more recently recognized that ARBs are neuroprotective and have potential therapeutic use in many brain disorders.

However, the complete pharmacological spectrum and therapeutic efficacy of individual ARBs have never been systematically compared, and the neuroprotective efficacy of these compounds has not been rigorously determined in controlled clinical studies.

MATERIAL AND METHODS

Animals

Experimental animals of either sex weighing 150-200g were obtained from Animal House, K. M. College of Pharmacy, Madurai. The animals were housed in polypropylene cages at a controlled room temperature of 24°C, under 12 h light and 12 h dark cycle and given standard laboratory feed and water *ad libitum.*

The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee of K.M. College of Pharmacy.

Chemicals

Injection Haloperidol, were procured from the Pharmacy of Meenakshi Mission Hospital Pharmacy, Madurai. Valsarten is the gift sample received from Cabling point Pharmaceuticals Limited, Chennai.

Methods

Groups

After one week of acclimatization, the experiment animals were divided randomly into 4 groups (n = 6). They were treated with test, standard drugs and saline (control) as per the schedule shown below.

Group I: Received normal saline orally as placebo for control.

Group II: Negative control received only injection Haloperidol intraperitoneally in the dose of 2mg/kg daily for 7 days.

Group III: Standard received L-Dopa and Carbidopa (100+25mg kg-1 p.o.) and Haloperidol intraperitoneally in the dose of 2mg/kg 45 minutes later daily for 7 days.

Group IV: Received Valsarten orally followed by Injection Haloperidol intraperitoneally in the dose of 2mg/kg 45 minutes later daily for 7 days.

The following parameters were recorded in all the rats of all 4 groups at day-0 before administration of any medicine, 4th day, 8th and 14th day after administration of medicines[4,5].

Assessment of Akinesia/ hypokinesia

Akinesia/ hypokinesia was Measured using Actophotometer. This test measures the exploration and the voluntary locomotion within an enclosed area. The objective value for the spontaneous motor

activity was obtained using a photoactometer (INCO Ltd., India). The animal was placed individually into a 30 cm × 30 cm black metal chamber with a screen floor and a light-tight lid. Six beams of red light were focused 2 cm above the floor into photocells on the opposite side. Each beam interruption was registered as an event on the external counter. The light beam breaks were counted for 5min.

Assessment of muscle activity: Rota Rod Test

The main symptom of the Parkinsonism disease is muscle rigidity. This effect can be easily studied in animal by using rotarod apparatus. Turn on the rotarod. Select the speed (20-25 rpm is ideal). Before the test, each animal was given 1 min exposure to the moving rod. The animal was placed on the rotating rod for 3mins. Latency to fall off from the rotating rod of animal in control and the treated group was recorded. Movement impairement was indicated by the inability of the animal to remain on the rotating rod for a 3min test period.

Assessment of Catatonia

It was assessed in terms of the time for which the mouse maintained an imposed position with both front limb extended and resting on a 4cm high bar (1cm diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut off time 300 seconds was applied. Between the determinations, the animals were returned to their individual home cages. All the observations were made between 9.00 and 15.00hrs in a quiet room at 23-25^{0}C.

Catatonia Scoring Method

If the animal maintained the imposed posture for at least 30seconds.It was considered to be cataleptic and the time was recorded in seconds. The animals were tested on every first, fourth and seventh day of the drug treatment and only the greater duration of the immobility were

Statistical Analysis

The results were tabulated and analyzed using Graph Pad Prism-6. One way ANOVA and Kruscal-Wallis tests for statistical significance and Dunn's multiple comparison test were used to compare within and between groups considering p-value of less than 0.05 as statistically significant.

RESULTS AND DISCUSSION

Locomotor activity of vehicle treated control group (Group I) was found to be 231-264 counts for all the two weeks of treatment.

Then there was a significant decrease in the locomotor activity of Haloperidol treated negative control animals (Group II) when compared to control group (Group I). Animals treated with Haloperidol (Group II) alone for 14 days showed a significant decrease on 4th day, 7th day and increase on 14th day in the locomotor activity when compared to control group (Group I).

Animals treated with standard drug (Group III)showed a nonsignificant variance in locomotor activity on the 0th day (156.58) which then significantly readings decreased to 141.8 and 135.4 in locomotor activity on the 4th and 7th day respectively and on the 14th day it increase to 204.45.

Animals treated with Valsarten (Group IV) showed a nonsignificant difference in the 0th day (185.35) and then a gradually increase in locomotor activity on the 4th and 7th day respectively (165.23 ± 21.8 and 178.58), on the 14th day it further increase to (198.34).

Motor activity slowly returned almost to the basal level on the 14th day in all the groups but not completely reverted. The present study showed that the Valsarten has significant protection in Haloperidol induced hypolocomotion.

The mean fall-off time of vehicle treated control group (Group I) animals from the Rotarod was found to be 32.58 to 38.23 seconds during weekly observation of the treatment.

Animals treated with Haloperidol (Group II) alone for 14 days showed a nonsignificant decrease on 4th day, significant (26.54), decrease on 7th day (20.13) and further increase (23.58) on 14th day in the latency off all when compared to normal group.

Animals treated with Standard drug and Haloperidol (Group III) for 14 days showed a

nonsignificant slightly decreases and increase (29.23) on 14th day in the latency of fall when compared to negative control group [Table No.3].

In Valsartan drug treated group (Group IV), a significant increases in fall off time was seen on 4th, 7th and 14th day as compared to the Haloperidol treated group (Group II). Fall off time was more in control group compared to others. The increased motor rigidity was same in all the groups on 7th day one day after stopping the Parkinson inducing drug haloperidol. No significant difference was observed between the treated and untreated groups on 7th day. On 14th day much more recovery of muscle tone was noted in all the groups evidenced by rota rod. But complete recovery was not observed in all the experimental groups 7 days after stopping the standard and investigational drugs.

In Catalepsy test, the group which received only Haloperidol (Group II), significantly, increases catalepsy which was seen on 1st, 4th and 7th day as compared to the Normal group (Group I). In standard treated group (Group III), a significant decrease in catalepsy was seen on 4th and 7th day as compared to the Haloperidol treated group (Group II) and increase on 14th day of the treatment.

In Valsarten treated group (Group IV), a significant decrease in catalepsy was seen on 4th and 7th day as compared to the Haloperidol treated group (Group II) and increase on 14th day of the treatment. Whereas no significant difference in catalepsy was seen when Group VI compared to standard treated group (Group III).

Parkinson's disease is a chronic neurodegenerative disorder characterized by loss of dopamine neurons of the SNpc. The pathogenesis of PD includes oxidative stress, protein accumulation like a-synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitotoxicity. Among all, oxidative stress is a crucial pathological mechanism for PD. SNpc is more vulnerable to reactive oxygen species as it contains more amount of dopamine.

Free radical damage to the central nervous system (CNS) is due to its high oxygen utility, increased lipid content and inadequate antioxidant enzymes than compared to other tissue. The free radical generation in the brain influences gene expression, subsequently leading to apoptosis and neuronal death. In the brain, an array of cellular defense systems, i.e., enzymatic and non-enzymatic antioxidants exists to counterbalance the generation of reactive oxygen species. Studies show that prolonged treatment with antiparkinson drugs such as dopamine agonist, dopamine replenishment therapy, and monoamine oxidase inhibitors leads to severe side effects and decrease in the sensitivity for the therapy. Further, typical neuroleptic drugs such as chlorpromazine, haloperidol, and reserpine use in schizophrenia lead to decrease in dopamine content and state of catalepsy.

Valsartan were used in the Haloperidol model in rats; were found to be significant in reducing the catalepsy, increasing the locomotor activity (actophotometer), and increasing the muscle activity (Rotarod test) in a Haloperidol model of Parkinson in rats which *indicates Valsartan* has potential effects against Parkinson's disease-like symptoms produced in various experimental models. The cataleptic induction model by neuroleptics in rodents is the widely accepted model to test the extra pyramidal side effects of antipsychotic agents. Evidences indicate that drugs which produce or reduce the catalepsy in rodents might also show the same effects in human beings. The conversion of levodopa to dopamine in serotonin neurons was proved as a compensatory measure in PD. In the present study confirm the effect Valsartan in Haloperidol induced Parkinson disease in experimental animals.

Table No.1: Locomotor activity of animals

S.No	Groups	0 day	4th day	7th day	14th day
1	Control	231.23 ± 4.8	245.58±4.3	268.5±8.34	264.75±4.9
2	Negative control	168.52 ± 4.8	121.23 ± 6.3 [a]	92.31 ± 3.8 [a]	164.7 ± 4.6 [a]
3	Standard Levodopa	156.58±5.2	141.8±3.62 [y]	135.40±6.4 [x]	204.45±4.58 [x]
4	Valsartan	185.35±5.6	165.23±2.8 [y]	178.58±6.5 [x]	198.34±6.6 [x]

Values were expressed as Mean ± SEM (n= 6)

a = ∗ ∗ ∗ ($P < 0.001$), b = ∗∗ ($P < 0.01$), and c = ∗ ($P < 0.05$) when compared with control group. x = ### ($P < 0.001$), y = ## ($P < 0.01$), and z = # ($P < 0.05$) when compared with negative control group.

Table No.2: Muscle rigidity of animals

S.No	Group	0 day	4th day	7th day	14th day
1	Control	38.23±0.6	36.45±0.91	30.23±0.23	32.58±1.4
2	Negative control	28.54±1.3	26.54±0.5[a]	20.13±1.3 [a]	23.58±0.9 [a]
3	Standard	26.78±0.39	24.23±1.2 [x]	22.45±1.5 [x]	29.23±0.8 [y]
4	Valsartan	30.14±1.8	28.56±0.5 [y]	29.53±0.4 [x]	31.25±1.2 [y]

Values were expressed as Mean ± SEM (n= 6)

a = ∗ ∗ ∗ ($P < 0.001$), b = ∗∗ ($P < 0.01$), and c = ∗ ($P < 0.05$) when compared with control group. x = ### ($P < 0.001$), y = ## ($P < 0.01$), and z = # ($P < 0.05$) when compared with negative control group.

Table No.3: Catalepsy- Scoring

S.No	Groups	0 day	4th day	7th day	14th day
1	Control	15.28 ± 1.5	10.12±1.8	17.54±0.9	12.47±0.8
2	Negative control	18.54 ± 1.8	20.34 ± 0.7 [a]	14.23± 0.9 [a]	18.71 ± 0.4 [a]
3	Standard Levodopa	14.25±0.5	13.47±0.6 [y]	16.28±0.3 [x]	17.64±1.12 [x]
4	Valsartan	19.24±0.3	17.58±0.8 [y]	20.58±0.3 [x]	21.38±05 [y]

Values were expressed as Mean ± SEM (n= 6)

a = ∗ ∗ ∗ ($P < 0.001$), b = ∗∗ ($P < 0.01$), and c = ∗ ($P < 0.05$) when compared with control group. x = ### ($P < 0.001$), y = ## ($P < 0.01$), and z = # ($P < 0.05$) when compared with negative control group.

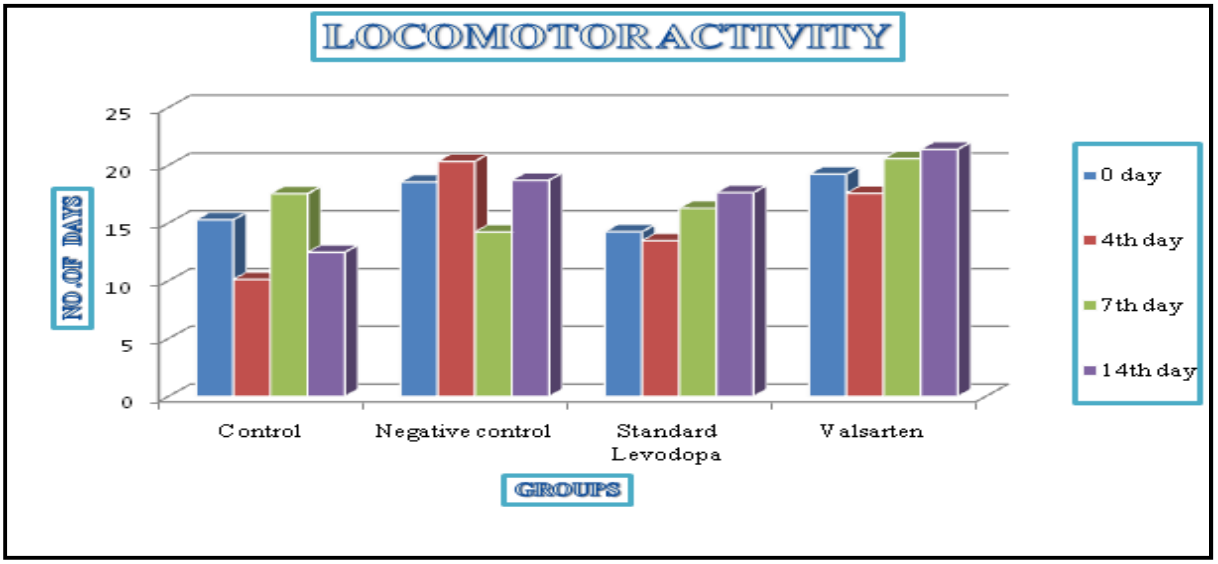

Figure No.1: Locomotor activity of animals was evaluated using Actophotometer

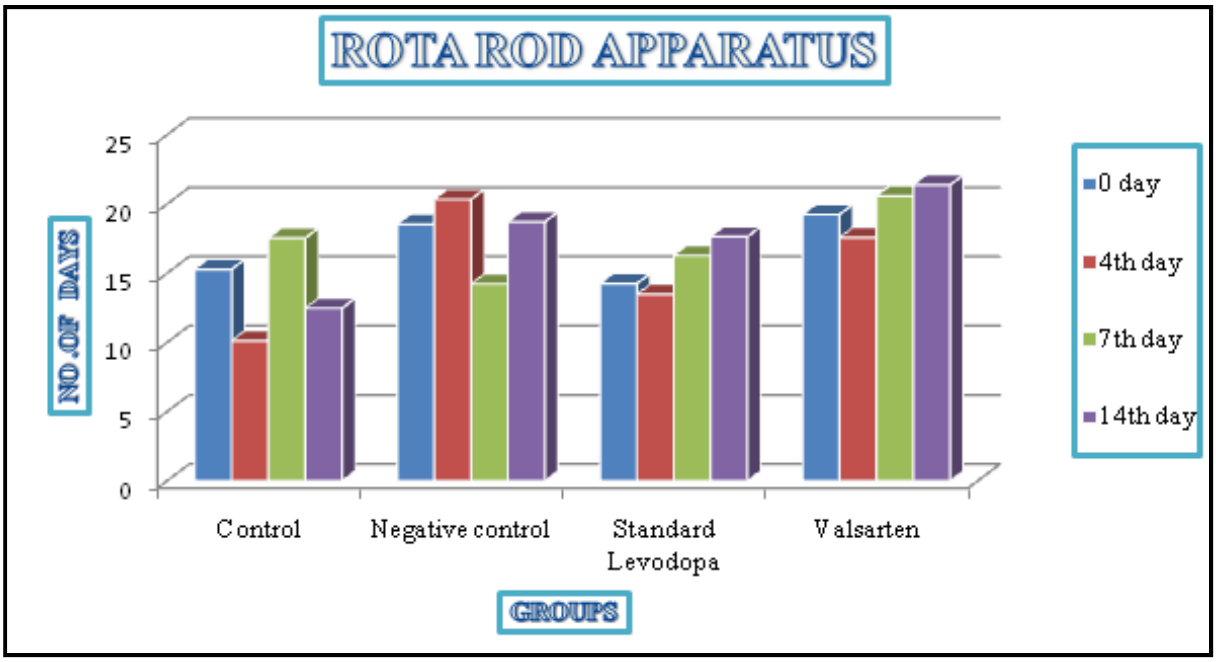

Figure No.2: Muscle Rigidity of animals was evaluated by using the Rotarod apparatus

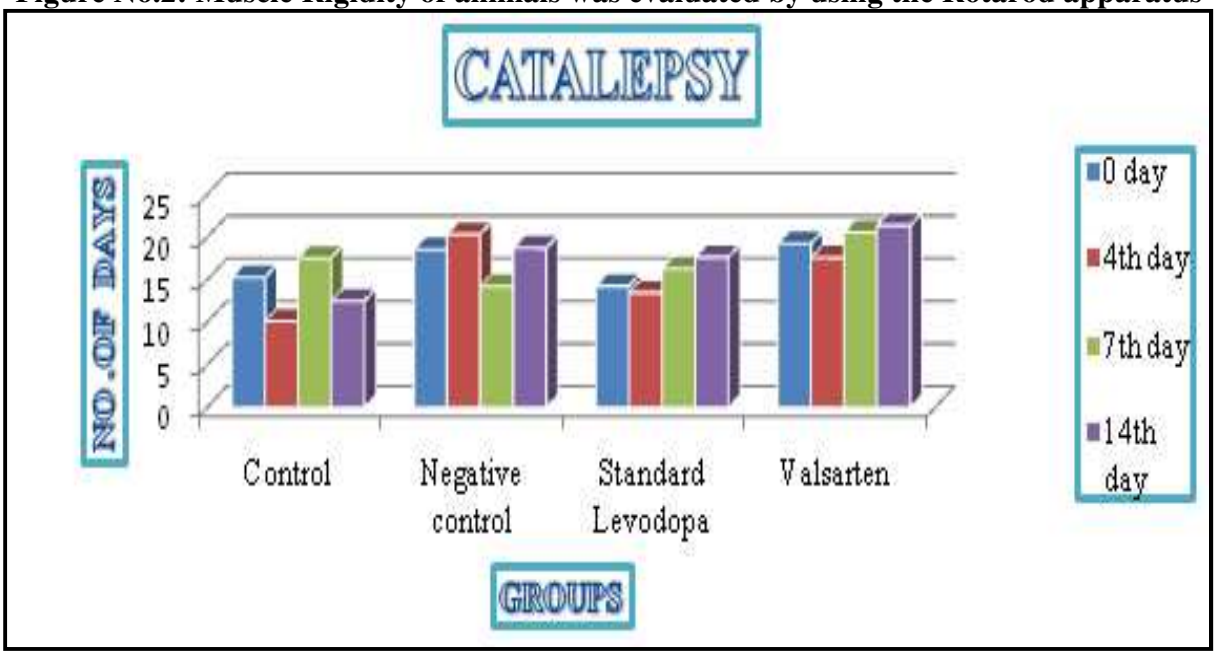

Figure No.3: Catalepsy- Scoring

CONCLUSION

Parkinson's disease is a progressive neurodegenerative disease accompanied by preferential loss of dopaminergic neurons of the substantia nigra pars compacta. Haloperidol by blocking D_2 receptors is commonly used to create experimental model of Parkinson's disease. The results of the present study conclusively showed that Valsartan has promising effect in animals with Parkinson's disease. And we appreciate further detailed molecular studies with this drug in anti-Parkinson's pharmacology and toxicology for neuroprotective effect.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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