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Evaluation of Antiparkinson Activity of Vitamin C, Tizanidine and Pregabalin against Haloperidol Induced Parkinsonism in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study deals with the antiparkinson effect of Levodopa/carbidopa, Vitamin C, Tizanidine, Pregabalin on haloperidol (1 mg/kg i.p) induced Parkinsonism in rats. Neural degeneration was induced in animals (Group II, III, IV, V, VI, VII, VIII) by i.p. administration of haloperidol at 1mg/kg. Positive control (Group-III) treated by levodopa/carbidopa (125 mg/kg p.o.), Test 1 (Group -IV) treated by vitamin C (120mg/kg p.o.), Test 2 (Group-V) treated by Tizanidine (0.3 mg/kg i.p.), Test 3 (Group -VI) treated by Pregabalin (30 mg/kg p.o.), Test 4 (Group-VII) treated by Vitamin C(120 mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) and Test 5 (Group-VIII) treated by Vitamin C(120 mg/kg p.o.) and Pregabalin (30mg/kg p.o) daily were given before 30 min of haloperidol challenged for 14 days. Behavioural parameters were recorded on day 14 for high bar test, actophotometer test, rotarod test. MDA level and Dopamine level were also recorded. Test 4 (Group- VII) showed more significant reduction in cataleptic score. In actophotometer, Test 4 (Group-VII) showed significant

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increase in locomotor activity. In rotarod test, Test 2 (Group-V) and test 4 (Group-VII) showed more significant increase motor coordination activity. Levodopa/Carbidopa showed more significant increase in dopamine level and significantly abolished cataleptic score. Vitamin C showed more significant decrease in MDA level. It can be concluded that the tizanidine and pregabalin may possess antiparkinsonian activity. Vitamin C and tizanidine may possess synergestic effect. These results showed that tizanidine and pregabalin may be used to improve catalepsy in Parkinson disease.

Keywords: Parkinson disease; haloperidol; levodopa/carbidopa; vitamin C; tizanidine; pregabalin.

1. INTRODUCTION

James Parkinson described Parkinson's disease (PD) as a chronic progressive neurological disorder for the first time in 1817 [1]. Parkinson's disease (PD), the second most common neurodegenerative disorder, is characterised by impaired motor coordination (muscle rigidity, tremor, postural imbalance, and movement slowness) as well as non-motor symptoms (mood disorders, sleep disorders, and cognitive deficit) [2]. 10 million people worldwide are afflicted with Parkinson's disease. It affects 2-3% of adults over the age of 65 [3]. Parkinson's disease (PD) is characterised principally by the degeneration of dopamine-carrying neurons in the substantia nigra, as well as extrapyramidal symptoms such as tremors, bradykinesia, rigidity, and inability to maintain normal posture [4]. Neuronal death in Parkinson's disease is caused by free radical damage, which results in the creation of Lewy bodies [5].

In general, a combined therapy of synthetic medications is more beneficial in the treatment of Parkinson's disease. Levodopa used in primary treatment for Parkinson's disease, although long-term use causes numerous negative effects [6]. Neurodegeneration in Parkinson's disease accelerates due to dopaminergic loss, oxidative stress caused by -synuclein aggregation (Lewy body), and mitochondrial failure. However, the precise aetiology of this chronic multifaceted neuronal illness remains unknown; genetic mutation, environmental variables, and age are the major causal factors in PD [7]. Prolonged use of antipsychotic medicines, such as haloperidol, produces dopamine receptor blockage in the corpus striatum, resulting in extrapyramidal manifestations comparable to Parkinson's disease. Catalepsy caused by neuroleptic medications such as haloperidol is an extensively used animal model for drug testing for Parkinson's disease. Catalepsy is the reduction in an animal's capacity to restore its externally imposed position.

Haloperidol, an antipsychotic that acts as a D2 receptor antagonist. In rodents, systemic treatment of haloperidol can cause catalepsy, a bradykinesia and rigidity characterised by the animal's inability to modify externally imposed postures. Catalepsy caused by the blocking of D2 dopaminergic receptors in the nigrostriatal pathway and is frequently utilised as an animal model for studying motor deficits seen in parkinsonian illnesses as well as screening possible antiparkinsonian compounds [8]. In Parkinson disease oxidative stress can be occurred. The Vitamin C as an antioxidant can provides protection against oxidative stress induced cellular damage by scavenging of reactive oxygen species [9]. The level of vitamin C in the brain is rarely impacted by external supply, but if it is consumed in the brain. increasingly owing to an oxidative assault [10]. Tizanidine is an alpha-2 adrenergic agonist, which means that it binds to and activates these receptors. Tizanidine reduces the release of norepinephrine, a neurotransmitter involved in nerve signal transmission, by activating alpha-2 adrenergic receptors. Norepinephrine has been found to play a role in muscle tone and spasticity regulation. It is most effective in muscle relaxation [11].

The pregabalin is antiseizure and antinociceptive drug. Pregabalin is a voltage-gated Ca2+ channel antagonist that exclusively binds to the alpha-2-delta subunit to provide antiepileptic and analgesic effects. Pregabalin's mode of action for pain relief: Pregabalin inhibits the VGCC, reducing glutamate and sensory neuropeptide release at the synapse via decreasing Ca2+ [12].

2. MATERIALS AND METHODS

2.1 Animals

For this study, 48 Wister albino rats either of sex weighing about 200-250gm were used. These rats were procured from registered (YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Satara)

breeder and were acquainted in the quarantine area for one week. After acquaintation, animals were transferred to the YSPM'S Yashoda Technical Campus ,Faculty of Pharmacy, Satara standard laboratory conditions of 22±2°C temperature., 50±15% of relative humidity, 12 hr dark/12hr light cycle and the animals had free access to pellets and water. The experiment was performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.2 Drugs and Chemicals

Table 1. Drugs and chemicals

Sr. No	Drugs/Chemicals	Manufacture/Suppliers
1	Haloperidol	Vamsi pvt. ltd, Solapur
2	Levodopa/carbidopa	Sun pharma laboratories ltd
3	Vitamin C	Joshi Agrochem Pharma Pvt. Ltd.
4	Tizanidine	Niksan Pharmaceutical
5	Pregabalin	Glenmark pharmaceutical ltd
6	Normal saline	Helios pharmaceuticals

2.3 Experimental Design

All animals were randomly selected into 8 groups containing 6 animals in each group. Group-I (Normal Control) was administered with Vehicle/Saline once a day for two weeks. All groups of animals were given haloperidol (1mg/kg i.p) once a day for two weeks except Group-I animals. Group-III (Standard control) was administered with levodopa/carbidopa (125mg/kg p.o.) once a day for two weeks. Group-IV (Test-1) was administered with Vitamin C (120mg/kg p.o.) once a day for two weeks. Group-V (Test 2) was administered with tizanidine (0.3mg/kg i.p.) Once a day for two weeks. Group-VI (Test 3) was administered with pregabalin (30mg/kg p.o.) once a day for two weeks. Group-VII (Test 4) was administered with combination of Vitamin C (120mg/kg p.o.) and Tizanidine (0.3mg/kg i.p.) once a day for two weeks. Group-VIII (Test 5) was administered with combination of Vitamin C (120mg/kg p.o.) and Pregabalin (30mg/kg i.p.) once a day for two weeks in a respective animals group. After the treatment for 14 days, all 8 groups of animals underwent the behavioural assessment tests includes Catalepsy test (High bar test). Locomotor activity using actophotometer test and Rotarod test (fall of time); also performed estimation of oxidative stress (MDA level) and evaluation of neurochemical test (dopamine level) [13,14,15].

2.4 High Bar Test

Haloperidol (1.0 mg/kg i.p) was used to induce catalepsy, and rats were examined every 30 minutes for up to 120 minutes using a standard bar test. Among the antipsychotics, haloperidol has a modest cataleptic effect. Catalepsy was graded in terms of severity. The imposed position of the front limbs on a 3 and 9 cm high wooden bar 1 cm wide. The end point is when both front paws are removed from the bar or the animal moves its head. All measurements were taken in a calm environment at 23-25 °C. Cataleptic animal maintaining this position for a period of time dependent upon the degree of catalepsy. If the animal maintained the imposed posture for at least 20 sec it was said to be cataleptic and time was recorded in sec. Animals maintaining the cataleptic posture from 0s to 10s scored 0;10s to 30s =1;30 s to 1 min =2;1 min to 2min =3;2min to 3min4 ;3 min to ∞ = 5. Animals were tested for catalepsy 0.5,1.0,2.0 and 3.0 hours after haloperidol administration [16,17].

2.5 Actophotometer Test

Any physical action that requires the animal to move from one location to another is known to as a locomotor talent. It is simple to assess with an actophotometer (activity cage). Actophotometer are used to detect hypokinesia or akinesia. The device consists of a cage with six light beam transmitters, and the receiver is situated so that only one photo beam is interrupted at a time when the animal crosses the one photo beam. When the photo beam is transferred to the acceptor photocell, one cell is completed. When the animal interrupted the beam light, the change was recorded. Total photo beam interruption in 10 minutes per animal was counted as activity [18,19].

2.6 Rota Rod Test

A horizontal metal rod (6 cm in diameter) is attached to a variable-speed motor in the apparatus. A partition disc (10.5 cm in diameter) divides the rod into five parts. The rod was positioned at a height of 50 cm to animals from jumping off the spinning rod. The animals were trained on the rotarod at a set speed of 20 rpm for 24 hours before the studies until they could stay on the equipment for 300 seconds without falling. Prior to the evaluation, all the rats went through five trials. The control rat remains attached to the rod for roughly 5 minutes. The treated rats were placed on the revolving rod at regular intervals, and the fall-off time was recorded [20,21].

2.7 Biochemical Evaluation

Assessment of Dopamine: The dissected brain samples were weighed, refrigerated at 80°C until assay, and homogenised in one millilitre of ice-cold0.1mmol/L perchloric acid solution containing 0.2 µg/ml L-isoproterenol hydrogen and 0.1mmol/L ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were centrifuged at 15,000 g for 30 minutes at 4°C, and the supernatant was filtered and kept at −80°C until the assay. Dopamine and DOPAC levels were determined using high performance liquid chromatography with an electrochemical detector and a 25 cm × 0.5 cm I.D column. The resulting sample peak is compared to the reference peak and given in micrograms per gram of tissue weight [22].

2.8 MDA Level

MDA, a marker of lipid peroxidation, was quantified spectrophotometrically using the Colado et al. (1997) technique and 1, 1, 3, 3-tetraethoxypropane as a standard. MDA is measured in n moles per mg protein. 500 l of phosphate buffered tissue homogenate (pH 7.4), 300 l of 30% trichloroacetic acid (TCA), 150 l of 5 N HCl, and 300 l of 2% w/v 2-thiobarbituric acid (TBA) were added, followed by 15 minutes at 90 °C heating. For 10 minutes, the mixture was centrifuged at 12,000 g. A pink supernatant was produced and spectrophotometrically quantified at 532 nm [23].

2.9 Histopathology

The animal was anaesthetized with chloroform, and the right hippocampus was fixed by immersion in a 10% neutral buffered solution. Transcranial perfusion with a fixation solution comprising 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3 was used to fix the brain. tissue was then embedded, sliced to a 5um thickness, and stained with hematoxyline and eosin for histological evaluation [24].

3. RESULTS

3.1 Cataleptic Activity

Negative Control (Group-II) Haloperidol (1mg/kg i.p.) showed significant elevation in the cataleptic score as compared to normal control (Group-I) animals. Group-III animals treated by Levodopa/ Carbidopa (125 mg/kg p.o.) for 14th day showed significant reduction in cataleptic score as compared to negative control (Group-II). Test 1(Group- IV) treated by Vitamin C (120 mg/kg p.o.) for 14th day did not show significant reduction in cataleptic activity as compared to negative control (Group-II). Test 2 (Group-V) treated by Tizanidine (0.3 mg/kg i.p.) for 14th day showed significant reduction in cataleptic activity as compared to negative control (Group-II). Test 3(Group-VI) treated by Pregabalin (30 mg/kg p.o.) for 14th day showed significant reduction in cataleptic activity as compared to negative control (GroupII)). Test 4 (Group-VII) treated by Vitamin C (120 mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) for 14th day showed more significant reduction in cataleptic activity as compared to negative control (Group-II). Test 5 (Group-VIII) treated by Vitamin C (120 mg/kg p.o.) and Pregabalin (30 mg/kg p.o.) for 14th day showed significant reduction in cataleptic activity as compared to negative control (Group-II) (Table 2).

3.2 Actophotometer

Negative Control (Group-II) with Haloperidol (1mg/kg i.p.) administration showed significant decrease in locomotor activity in comparison to normal control (Group I). Group-III treated by Levodopa/Carbidopa (120mg/kg p.o.) for 14th day showed more significant increase in locomotor activity as compared to negative control (Group II). Test 1 (Group-IV) treated by Vitamin C (120mg/kg p.o.) for 14th day showed significant increase in locomotor activity as compared to negative control (Group-II). Test 2 (Group-V) treated by Tizanidine (0.3mg/kg i.p.) for 14th day showed significant increase in locomotor activity as compared to negative control (Group II). Test 3 (Group-VI) treated by Pregabalin (30 mg/kg

p.o.) for 14th day showed significant increase in locomotor activity as compared to negative control (Group-II). Test 4 (Group- VII)) treated by Vitamin C (102mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) for 14th day showed significant increase in locomotor activity as compared to

Table 2. Effect of vitamin C, tizanidine and pregabalin on haloperidol induced catalepsy at 14th day

Group	Treatment (Dose)	Cataleptic Score			
		30 min	60 min	120 min	180 min
Group-I (Normal control)	--------	0±0.0	0±0.0	0±0.0	0±0.0
Group-II (Negative control)	Haloperidol (1 mg/kg)	2.66 ±0.13*	2.68 ±0.20*	2.50 ±0.30*	2.89 ±0.11*
Group-III (Positive control)	Haloperidol (1 mg/kg)+ Levodopa/carbidopa (125 mg/kg)	0.00 ± 0.00**	0.00 ± 0.00**	0.00 ± 0.00**	0.00 ± 0.00**
Group-IV (Test 1)	Haloperidol (1 mg/kg)+ Vitamin C(120 mg/kg)	2.33 ± 0.11**	2.33 ± 0.00**	2.31 ± 0.03**	2.66 ± 0.23**
Group-V (Test 2)	Haloperidol (1 mg/kg)+ Tizanidine(0.3 mg/kg)	0.33 ± 0.02**	0.63 ± 0.01**	0.33 ± 0.02**	0.33 ± 0.01**
Group-VI (Test 3)	Haloperidol (1 mg/kg)+ Pregabalin(30 mg/kg)	0.66 ± 0.05**	0.66 ± 0.01**	0.66 ± 0.02**	0.66 ± 0.04**
Group-VII (Test 4)	Haloperidol (1 mg/kg)+ VitaminC(120 mg/kg)+ Tizanidine(0.3 mg/kg)	0±0.0**	0.00 ± 0.00**	0.00 ± 0.00**	0.00 ± 0.00**
Group-VIII (Test 5)	Haloperidol (1 mg/kg)+ VitaminC(120 mg/kg)+ Pregabalin(30 mg/kg)	0.70 ± 0.07**	0.83 ± 0.22**	0.22 ± 0.78**	0.12 ± 0.33**

Data are expressed as Mean ±SEM (n=6) NC=Negative Control, PC=Positive Control
**P<0.01 Negative control compared with normal control group*
***p<0.01 Standard control and test control compared with negative control group*

Table 3. Effect of vitamin C, tizanidine and pregabalin on Locomotor activityin rats using actophotometer at 14th day

Group	Treatment (Dose)	Number of Locomotor activity in 10 min
Group-I (Normal control)	----------	399 ± 41.2
Group- II (Negative control)	Haloperidol(1 mg/kg)	170 ± 21.3*
Group- III (Positive control)	Haloperidol(1 mg/kg) + Levodopa/carbidopa(125 mg/kg)	341 ± 33.3**
Group- IV (Test 1)	Haloperidol(1 mg/kg) + Vitamin-C(120 mg/kg)	230 ± 30.5**
Group -V (Test 2)	Haloperidol(1 mg/kg) + Tizanidine(0.3 mg/kg)	231 ± 27.82**
Group- VI (Test 3)	Haloperidol(1 mg/kg) + Pregabalin(30 mg/kg)	201 ± 26.2**
Group- VII (Test 4)	Haloperidol(1 mg/kg) + VitaminC(120 mg/kg) + Tizanidine(0.3 mg/kg)	292 ± 24.6**
Group -VIII (Test 5)	Haloperidol(1 mg/kg)+ VitaminC(120 mg/kg)+ Pregabalin(30 mg/kg)	319 ± 28.7**

Data are expressed as Mean ±SEM (n=6) NC=Negative Control, PC=Positive Control.
**p<0.01 negative control compared with normal control group*
***p<0.01 Standard control and test control compared with negative control group*

negative control (Group-II). Test 5 (Group -VIII) treated by Vitamin C (120 mg/kg p.o.) and Pregabalin (30 mg/kg p.o.) for 14th day showed more significant increase in locomotor activity as compared to negative control (Group-II) (Table 3).

3.3 Rotarod Test

Negative Control (Group-II) with haloperidol administration showed a significant decrease in fall off time as compared to Normal Control (Group-I). Group-III treated by Levodopa /Carbidopa (120 mg/kg p.o.) for 14th day showed more significant increase motor co- ordination activity (fall-off time) as compared to negative control (Group- II).Test 1 (Group-IV) treated by Vitamin C (120 mg/kg p.o.) for 14th day did not show significant increase motor coordination activity (fall-off time) as compared to negative control (Group-II).Test 2 (Group-V) treated by Tizanidine (0.3 mg/kg i.p.) for 14th day showed significant increase in motor coordination activity (fall-off time) as compared to negative control (Group-II). Test 3 (Group-VI) treated by Pregabalin (30 mg/kg p.o.) for 14th day showed significant increase motor coordination activity (fall- off time) as compared to negative control (Group-II). Test 4(Group-VII) treated by Vitamin C (120 mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) for 14th day showed more significant increase motor coordination activity (fall-off time) as compared to negative control (Group-II). Test 5 (Group-VIII) treated by Vitamin C (120 mg/kg p.o.) and Pregabalin (30 mg/kg p.o.) for 14th day showed significant increase motor coordination activity (fall-off time) as compared to negative control (Group-II) (Table 4).

3.4 Dopamine Level

Negative Control (Group-II) Haloperidol (1mg/kg i.p.) showed decrease in hippocampal dopamine level compared with Normal Control (Group-I). Group-III treated by Levodopa/Carbidopa (120 mg/kg p.o.) for 14th day showed more significant increase in dopamine level compared to negative control (Group-II). Test 1 (G-IV) treated by Vitamin C (120 mg/kg p.o.) for 14th day did not show significant increase in dopamine level compared to negative control (Group-II). Test 2 (Group-V) treated by Tizanidine (0.3 mg/kg i.p.) for 14th day did not show significant increase in dopamine level compared to negative control (Group-II). Test 3 (Group-VI) treated by Pregabalin (30 mg/kg p.o.) for 14th day did not show significant increase in dopamine level compared to negative control (Group-II). Test 4 (Group-VII) treated by Vitamin C (120 mg/kg p.o.) and Tizanidine ((0.3 mg/kg i.p.) for 14th day did not show significant increase in dopamine level compared to negative control (Group-II).

Table 4. Effect of vitamin C, tizanidine and pregabalin on muscle co-ordination behaviour in rats at 14th day

Group	Treatment (Dose)	Motor co-ordination fall of time in sec
Group -I (Normal control)	----------	172 ± 1.80
Group - II (Negative control)	Haloperidol (1 mg/kg)	68 ± 7.0*
Group - III (Positive control)	Haloperidol (1 mg/kg)+ Levodopa/carbidopa (125 mg/kg)	154 ± 1.62**
Group- IV (Test 1)	Haloperidol (1 mg/kg)+ Vitamin-C (120 mg/kg)	86 ± 1.00**
Group -V (Test 2)	Haloperidol (1 mg/kg)+ Tizanidine(0.3 mg/kg)	122 ± 1.50**
Group -VI (Test 3)	Haloperidol (1 mg/kg)+ Pregabalin (30 mg/kg)	95 ± 9.80**
Group -VII (Test 4)	Haloperidol (1 mg/kg)+ Vitamin-C (120 mg/kg)+ Tizanidine(0.3 mg/kg)	125 ± 8.20**
Group- VIII (Test 5)	Haloperidol (1 mg/kg)+ Vitamin-C (120 mg/kg)+ Pregabalin (30 mg/kg)	98 ± 1.11**

Data are expressed as Mean± SEM (n=6) NC=Negative Control, PC=Positive Control
**p<0.01 Negative control compared with normal control group*
***p<0.01 Standard control and test control compared with negative control group*

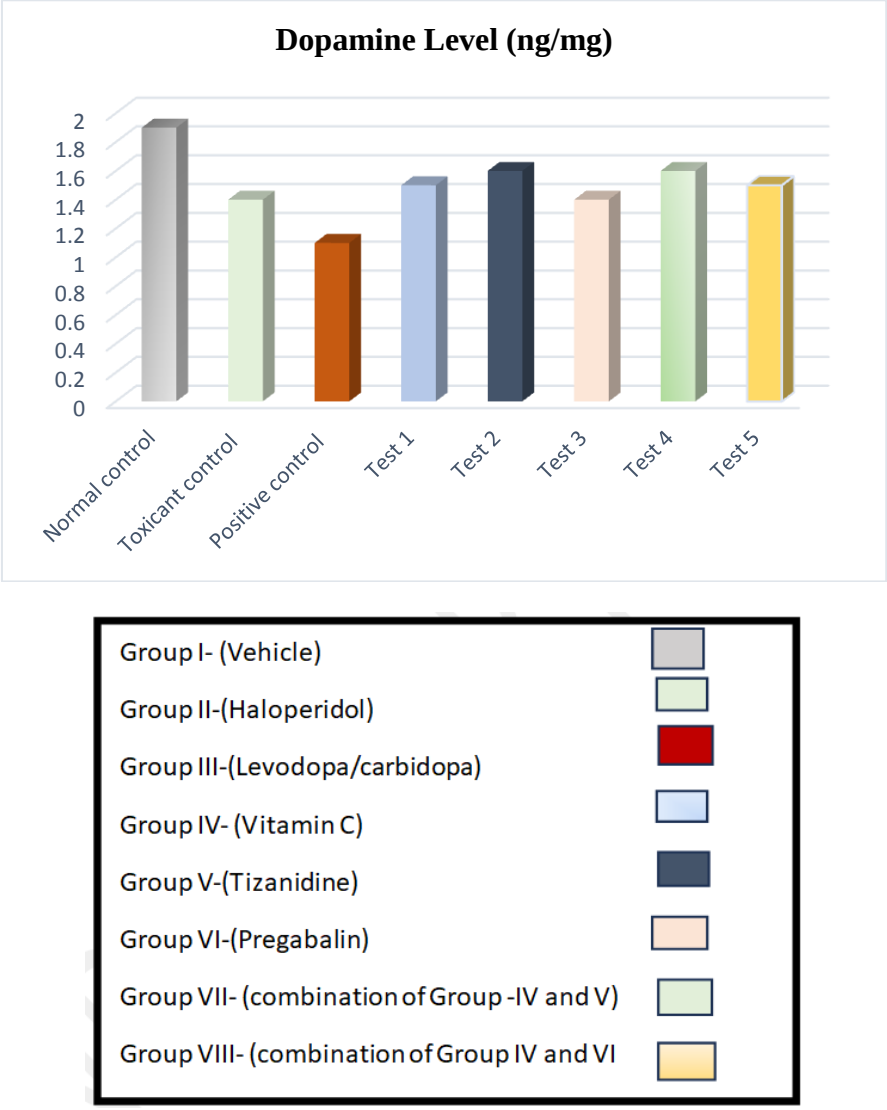


Fig. 1. Effect of Vitamin C, Tizanidine, Pregabalin treatment on neurotransmitters level in brain
*Data presented as mean ± SEM (n=6) normal control ***p<0.01, **p<0.01compared with negative control*

Test 5 (Group-VIII) treated by Vitamin C (120 mg/kg p.o.) and Pregabalin (30 mg/kg p.o.) for 14th day did not show significant increase in dopamine level compared negative control (Group-II) (Fig. 1).

3.5 MDA Level

Negative Control (Group-II) Haloperidol (1mg/kg i.p) caused a marked increase in lipid peroxidation as compared with normal control (Group-I). Group-III treated by Levodopa/Carbidopa (125mg/kg p.o.) for 14th day did not show significant decrease in MDA level as compared with negative control (Group-II). Test 1 (Group - IV) Vitamin C (120mg/kg p.o.) for 14th day showed more significant decrease in MDA level as compared to negative control (Group-II). Test 2 (Group-V) treated by Tizanidine (0.3mg/kg i.p.) for 14th day did not show significant decrease in MDA level as compared to negative control (Group-II). Test 3 (Group-VI) treated by Pregabalin (30mg/kg p.o.) for 14th day did not show significant decrease in MDA level as compared to negative control (Group-II). Test 4 (Group-VII) treated by Vitamin C (120mg/kg p.o.) and Tizanidine (0.3mg/kg i.p.) for 14th day showed highly significant decrease in MDA level as compared negative control (Group-II). Test 5 (Group-VIII) treated by Vitamin C (120mg/kg p.o.) and Pregabalin (30mg/kg p.o.) for 14th day showed significant decrease in MDA level as compared to negative control (Group-II).

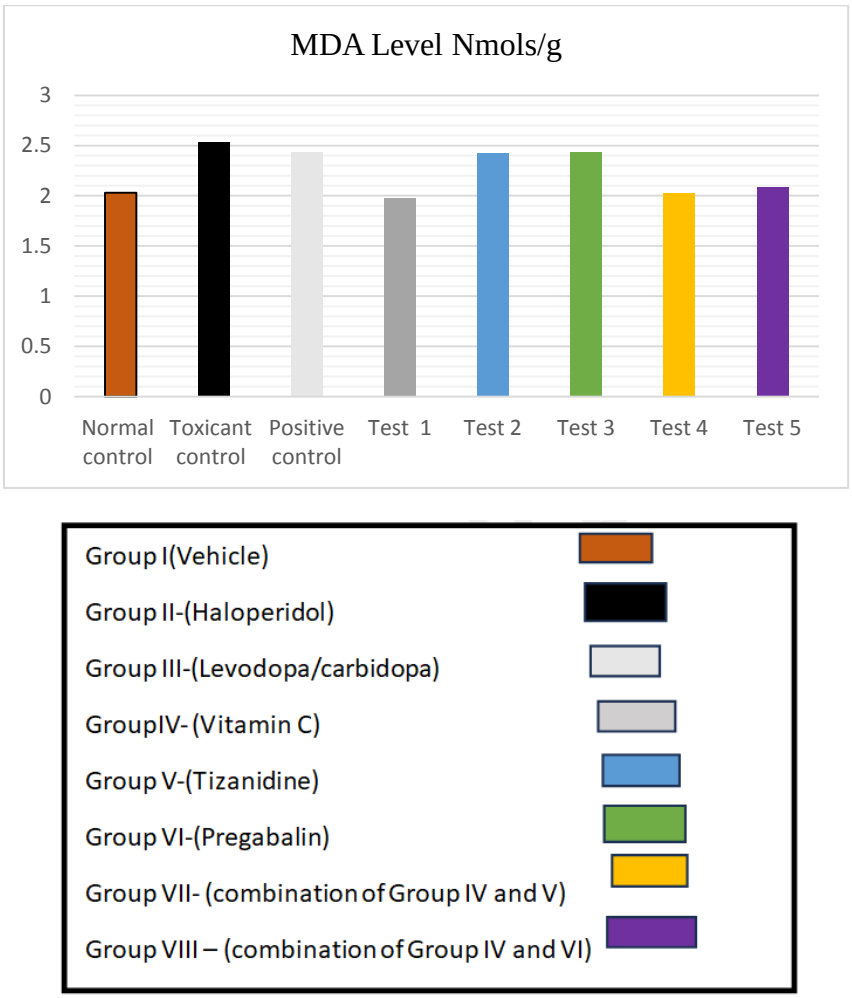
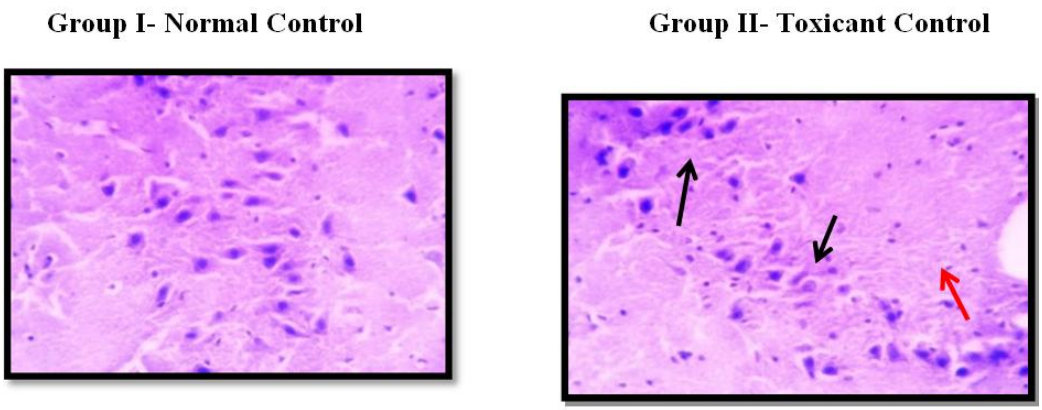
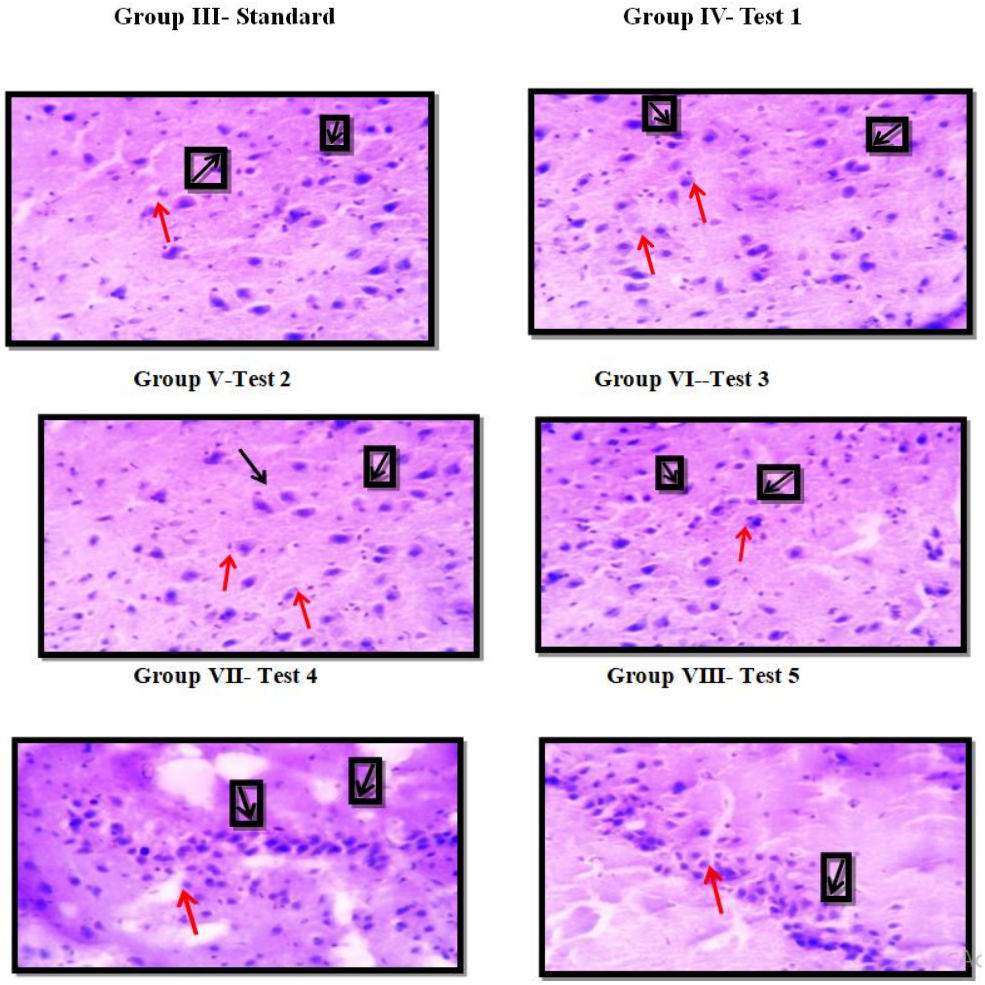


Fig. 2. Effect of Vitamin C, Tizanidine, Pregabalin treatment on the level of malondialdehyde (MDA) in the hippocampus in an animal model of parkinson's disease induced by haloperidol

*Data were expressed as mean ± SEM. for (n=6) in each group. normal control ***p<0.01, **p<0.01compared with negative control*

3.6 Histopathology





The slides of the brain tissue of rats were observed under 40x magnification for histopathological changes in the brain. Normal control group (NCG) (Group-I) showed intact cells. Brains of Negative Control (Group-II) Haloperidol (1mg/kg i.p.) rat showed necrotic neurons with intracellular spacing showing edema. The positive control (Group-III) Levodopa/Carbidopa (125mg/kg p.o.) showed mild necrosis with more intact cells. Test 1 (Group-IV) Vitamin C (120mg/kg p.o.) showed a little effect on neurodegeneration. Minimal changes in cell integrity were seen. Test 2 (Group-V) Tizanidine (0.3mg/kg i.p.) showed mild necrosis with intact cell. Test 3 (Group-VI) Pregabalin (30mg/kg p.o.) showed very little effect on neurodegeneration. Minimal changes in cell integrity were seen. Teat 4 (Group-VII) Vitamin C (120mg/kg p.o.) + Tizanidine (0.3 mg/kg i.p) showed very less disorganization of neuronal cells, and very few intracellular spacing was noticed. Test 5 (Group-VIII) Vitamin C (120mg/kg p.o.) + Pregabalin (30mg/kg p.o.) showed mild necrosis with intact cell.

4. DISCUSSION

We thoroughly investigated the potential of haloperidol-induced catalepsy as an animal model for parkinsonism. We demonstrated that the haloperidol-induced catalepsy paradigm has been consistently and successfully employed. The model has been used to gain a better understanding of the neurobiology behind parkinsonism and, more importantly, to seek for and develop new therapies [25]. The haloperidol-induced catalepsy model has face validity for motor deficits, particularly bradykinesia seen in Parkinson's disease and other associated disorders [26]. Muscle weakness in limbs with tremors or rigidity appears to be the primary sign of Parkinson's disease, which may be related to disrupted motor functioning caused by basal ganglia dysfunction [27]. According to research, long-term use of antiparkinson medicines such as dopamine agonists, dopamine replenishment therapy, and monoamine oxidase inhibitors causes severe adverse effects and a decline in therapy sensitivity [28].

Vitamin C can provide protection against oxidative stress induced cellular damage by scavenging of reactive oxygen species [29]. Tizanidine is an alpha-2 adrenergic agonist, which means that it binds to and activates these receptors. Tizanidine reduces the release of norepinephrine, a neurotransmitter involved in nerve signal transmission, by activating alpha-2 adrenergic receptors. Tizanidine is effective in muscle relaxation. It was giving significant effect in rota rod test [30]. Pregabalin is a voltage-gated Ca2+ channel antagonist that exclusively binds to the alpha-2-delta subunit to provide antiepileptic and analgesic effects. Pregabalin's mode of action for pain relief [31].

In the high bar test the cataleptic score was increased in haloperidol (1 mg/kg) administered rats as compared to normal control group. In the disease control (Group-II) there is significantly elevated catalepsy with sign increase in paw retention time on bar. Test 2 (Group-IV) treated by tizanidine (0.3 mg/kg) showed significant reduction in cataleptic activity 14th day. Test 1(Group-IV) treated by Vitamin C (120 mg/kg p.o.) did not show significant reduction in cataleptic activity at 14th day. Test 3(Group-VI) treated by Pregabalin (30 mg/kg p.o.) showed significant reduction in cataleptic activity at 14th day. Test 4 (Group-VII) treated by Vitamin C (120 mg/kg p.o.) + Tizanidine (0.3 mg/kg i.p.) showed more significant reduction in cataleptic activity at 14th day. Test 5 (Group- VIII) treated by Vitamin C (120mg/kg p.o.) +Pregabalin (30 mg/kg p.o.) showed significant reduction in cataleptic activity at 14th day according to this research work.

In locomotor activity by actophotometer was decreased in haloperidol (1 mg/kg) administered rats as compared to normal control (Group-I). Group-III treated by Levodopa/Carbidopa (120 mg/kg p.o.) showed significant increase in locomotor more significant increase in locomotor activity at 14th day. Test 2 (Group-V) treated by Tizanidine (0.3 mg/kg i.p.) can show significant increase in locomotor activity at 14th day. Test 3 (Group-VI) treated by Pregabalin (30 mg/kg p.o.) can show significant increase in locomotor activity at 14th day. Test 4 (Group-VII) treated by Vitamin C (102 mg/kg p.o.) + Tizanidine (0.3 mg/kg i.p.) did not show significant increase in locomotor activity. Test 5 (Group-VIII) treated by Vitamin C (120 mg/kg p.o.) +Pregabalin (30 mg/kg p.o.) show significant increase in locomotor activity at 14th day according tothis research work .

Haloperidol (1 mg/kg) showed a decrease in retention time on the Rota rod apparatus, which clearly shows the muscle co-ordination property dysfunction due to dopaminergic depletion. Group-III treated by Levodopa/Carbidopa (120 mg/kg p.o.) can increase motor coordination activity (fall-off time) at 14th day. Test 1 (Group-IV) treated by Vitamin C (120 mg/kg p.o.) did not showed significant increase motor coordination activity (fall-off time). Test 2 (Group- V) treated by Tizanidine (0.3 mg/kg i.p.) showed more significant increase motor coordination activity (fall-off time). Test 3 (Group-VI) treated by Pregabalin (30 mg/kg p.o.) show significant increase motor coordination activity (fall- off time) at 14th day. Test 4(Group-VII) treated by Vitamin C (120 mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) can show more significant increase motor coordination activity (fall-off time) at 14th day. Haloperidol (1mg/kg) treated animals shows decrease the dopamine. Group-III treated by Levodopa/Carbidopa (120 mg/kg p.o.) showed more significant increase in dopamine level. Group IV-VIII did not significantly increase dopamine level.

Administration with Haloperidol(1mg/kg) show increase the MDA level. Test 1 (Group-IV) Vitamin C (120 mg/kg p.o.) showed more significant decrease in MDA level.

5. CONCLUSION

Haloperidol is a more common way to induce a neural degeneration model in experimental animals. Haloperidol (1 mg/kg) treated animals showed increase cataleptic activity, decrease locomotor activity using actophotometer, decrease motor co- ordination (fall-off time), decrease dopamine level, increase MDA level. Test 4 (Group- VII) treated by Vitamin C (120 mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) showed more significant reduction in cataleptic activity. In actophotometer activity Test 4 (Group-VII) treated by Vitamin C (120 mg/kg p.o.) and Tizanidine (0.3 mg/kg i.p.) showed significant increase in locomotor activity. In rotarod test, Test 2 (Group-V) treated by Tizanidine (0.3 mg/kg i.p.) showed more significant increase motor coordination activity (fall-off time). Levodopa/Carbidopa (125 mg/kg p.o.) showed more significant increase in dopamine level. Vitamin C (120 mg/kg p.o.) showed more significant decrease in MDA level.

It can be concluded that the tizanidine and pregabalin may possess antiparkinsonian

activity. The Combination of Vitamin C and Tizanidine may possess Synergestic effect. These results showed that tizanidine and pregabalin may be used to improve catalepsy in PD. However further investigation is required to establish this neuroprotective response in another experimental animal model.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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