

Neuroprotective Effect of Piracetam and Vincamine in a Rat Model of Haloperidol-induced Parkinson's Disease

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Abstract

To evaluate the neuroprotective effect of nootropic drugs, piracetam and vincamine, on Parkinson's disease (PD) in rats, forty adult male Wistar albino rats were randomized into five equal groups: control, haloperidol-induced PD group, and PD groups orally given piracetam (300 mg/kg/day), vincamine (20 mg/kg/day) or both. Four weeks later, motor performance was assessed by stepping test. Y-maze, forced swimming and olfactory preference tests were done for cognitive and behavioral evaluation. Blood samples were collected for measuring serum glucose, calcium, creatine phosphokinase (CPK) and glial cell-derived neurotrophic factor (GDNF). Thereafter, rats were sacrificed and brains were dissected. Striatal tissue of left hemisphere was isolated and homogenized for assay of dopamine, malondialdehyde (MDA), nitrite/nitrate, reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), tumor necrosis factor-alpha (TNF- α) and interleukin-1beta (IL-1 β). Right hemisphere was used for histopathological examination of substantia nigra. Results: Rats of PD group showed bradykinesia, cognitive impairment, depressive-like behavior and hyposmia, reductions in serum calcium and GDNF, and in striatal dopamine, GSH, GPx and SOD, while serum glucose and CPK, and striatal MDA, nitrite/nitrate, IL-1 β and TNF- α were increased, as compared to control. Both drugs improved neurological dysfunction and biochemical parameters, as compared to PD group. The histopathological findings revealed neuro-degeneration and neuro-inflammation in PD group, that improved in treated groups. The piracetam effect was mainly anti-inflammatory, while vincamine was mainly antioxidant. Combined treatment resulted in a more potent amelioration of haloperidol-induced changes. Conclusion: Piracetam and vincamine exhibit neuroprotective activity in haloperidol-induced PD, that is more potent with their combination.

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder affecting over ten million people worldwide [1]. It is accompanied by progressive loss of dopaminergic neurons in the substantia nigra pars compacta, resulting in significant impairment of dopamine release [2]. Patients with PD are presented by motor and non-motor symptoms. The traditional motor symptoms include bradykinesia, tremor, rigidity and postural instability [3]. Non-motor symptoms of PD include olfactory dysfunction, memory impairment, sleep disorders, anxiety and depression [4].

The underlying causes of PD include environmental factors [5], genetic factors [6] and drug-induced ones. Haloperidol is a classic antipsychotic drug, which causes Parkinson-like syndrome and extrapyramidal symptoms [7]. It can be used to induce experimental model of PD through blockage of the dopaminergic D2 receptors [8].

Nootropic drugs may have a role in protection and amelioration of the associated symptoms of PD, being a neurodegenerative disease. The metabolic enhancer piracetam, a cyclic derivative of gamma-aminobutyric acid, was claimed to have neuroprotective effect in many neurological disorders, as it enhances membrane fluidity, cognitive function and neurotransmission [9]. Vincamine, a plant alkaloid, can be used clinically in the therapy of cerebral and circulatory disorders [10].

The aim of the present study was to investigate a neuroprotective effect of piracetam and vincamine on a haloperidol-induced rat model of PD, and whether or not their combined administration provides a better protection.

1. Materials and Methods.

1.1. Experimental Animals and Design.

All procedures were conducted in adherence to the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press), with the approval of the Ethics Committee of Faculty of Medicine, Menoufia University, Egypt. Forty adult male Wister albino rats, weighing 160-200 grams each, were used in this study. Rats were kept on standard laboratory rat chow and water ad libitum. They were housed in cages measured 70x70x60 cm, 4 animals/cage under normal light/dark cycle at room temperature throughout the study period. Animals were left for one week for acclimatization, then they were randomly divided into five equal groups (n=8): (i) Control (C) group, in which rats were given 0.5 ml of distilled water by oral gavage, and intraperitoneally (i.p) injected with 0.5 ml of normal saline, once daily for 4 weeks. (ii) PD group, where rats were given 0.5 ml of distilled water by oral gavage, once daily for 4 weeks, and i.p. injected with 5 mg/kg body weight of haloperidol (Nile Company for Pharmaceutical, Egypt) dissolved in 0.5 ml of normal saline, once daily for 4 weeks [11]. (iii) Piracetam-treated PD (PD+Pir) group, where rats were given 300 mg/kg body weight of piracetam (Amoun Company, Egypt) [12] dissolved in 0.5 ml of distilled water by oral gavage, once daily for 4 weeks, and they were i.p. injected with haloperidol as in PD group. (iv) Vincamine-treated PD (PD+Vin) group, where rats were given 20 mg/kg body weight of vincamine (Glaxo Company, Egypt) [13] dissolved in 0.5 ml of distilled water by oral gavage, once daily for 4 weeks, and they were i.p. injected with haloperidol as in PD group. (v) Combined

piracetam- and vincamine-treated PD (PD+Pir&Vin) group, where rats received piracetam as in PD+Pir group and vincamine as in PD+Vin group, and they were injected with haloperidol as in PD group.

After 4 weeks-experimental period, rats underwent neurological and behavioral assessment tests, and then blood samples were collected. Then, rats were sacrificed by injection of lethal dose of thiopental sodium. Immediately after sacrifice, rats were decapitated, and brains were carefully dissected. The basal ganglia of left hemispheres were isolated, and the striatal tissue was homogenized for biochemical analyses. The right hemispheres were preserved in 10% formalin to be used for histopathological studies.

1.2. Neurological and behavioral assessment.

Neurological and behavioral tests were done at room temperature in a calm room without any external interference.

1.2.1. Stepping test.

Stepping test was used to assess motor performance of rats. The rat was firmly suspended from its hindquarters, while it supported its weight on forelimbs. Then, the rat moved backward along the table (0.9 m in 5 seconds) three times repeatedly per session. For each session, the total calculated score was the sum of the number of adjusting steps observed in the three tests [14].

1.2.2. Y-maze test.

Y-maze is considered a quick and relatively simple test of spatial working memory. The Y-maze used was made of wood with three arms of equal size (60cm × 12cm × 25cm), converged at a 120° angle with each other. Each rat was placed at the center of the maze and permitted to move freely for exploration of the arms for 8 min-duration. An arm

entry was considered when the hind paws of the rat were completely within the arm. Any three successive entries of the three different arms were evaluated as a correct choice. Spontaneous alternation refers to the natural tendency of rodents to spontaneously choose alternate arms in a Y-maze. The percentage of spontaneous alternation was calculated as follows: (actual alternations/maximum alternations) × 100. The number of maximum alternations equals total number of arms entered minus 2 [15].

1.2.3. Forced swimming test.

Rats were individually placed in cylinder (15cm in diameter and 40cm in height), containing water. Immobility time and latency to immobility were evaluated during 5 min-period. Immobility was assigned when no additional activity observed other than that required to keep the rat head above the water. Depressive-like behavior was defined as an increase in the time of immobility and a decrease in latency to immobility [16].

1.2.4. Olfactory preference test.

The rats were presented small aliquots of either a novel scent (vanilla) or water simultaneously. Time spent for sniffing each odor is recorded for three minutes [17].

1.3. Biochemical analysis.

1.3.1. Blood sampling and analysis.

Fasting blood samples were collected from the retro-orbital venous plexus, using fine heparinized capillary tubes introduced into medial epicanthus of the rat's eye under light halothane inhalation anesthesia. The blood samples were left to clot for 15 minutes at room temperature. Then, they were centrifuged at 3000 r.p.m for 15 minutes, and the serum was separated and stored at -20°C until used. Serum glucose and calcium levels was

estimated using their specific kits (Biodiagnostic Company, Egypt). Serum creatine phosphokinase (CPK) level was measured using specific kit purchased from Biovision Incorporation (USA), according to manufacturer's instructions. Serum glial cell-derived neurotrophic factor (GDNF) level was measured using rat ELISA kit (My BioSource Incorporation, USA) and following the manufacturer's instructions.

2.3.2 Striatal tissue homogenization and biochemical assay.

Immediately after sacrifice rats were decapitated. Brains were carefully removed and washed with ice-cold saline. The basal ganglia of the left hemisphere of each brain were isolated, and the striatal tissue was identified, weighed, and homogenized in 10% (w/v) phosphate-buffered solution (PH=7), using glass rod. The homogenate was centrifuged at 2000×g for 10 min, and the supernatant was kept at -80°C until biochemical analyses.

Striatal dopamine level was measured using a double-antibody sandwich ELISA kit (Sunred Company, Shanghai, China), according to the manufacturer's instructions. Striatal malondialdehyde (MDA) level was measured using its specific kit purchased from Biodiagnostic Company, Egypt. Nitrite/nitrate level in striatal tissue was estimated using colorimetric kit purchased from Biodiagnostic Company, Egypt, and following the manufacturer's instructions.

Striatal TNF- α and IL-1 β levels were measured using the corresponding rat ELISA kits purchased from Sunred Company (Shanghai, China) and My BioSource Incorporation (USA), respectively, and following the manufacturer's instructions.

Striatal homogenate reduced glutathione (GSH) level was measured by its colorimetric kit purchased from Biodiagnostic Company (Dokki, Giza, Egypt). Striatal glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzymatic activities were determined using their specific colorimetric kits (Biodiagnostic Company, Egypt), according to the manufacturer's instructions.

1.4. Histopathological examination.

Immediately after dissection, the right hemisphere of each rat brain was placed in 10% (v/v) formalin for 24 hours. Tissues were washed in tap water, and serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Thereafter, specimens of substantia nigra pars compacta (SNpc) were cleared in xylene for 20-30 minutes and impregnated in soft paraffin wax for two hours, then in hard paraffin wax at room temperature. Finally, tissue blocks were formed and cut out into sections of five microns thickness, and then stained with hematoxylin and eosin stain (H&E) [18]. After staining, the sections were examined and photographed using a bright-field microscope.

1.5. Statistical analysis.

All results were expressed as the mean \pm standard deviation (SD). The data were analyzed using SPSS program version 22.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA, the F test, and post hoc Tukey's multiple comparison test were calculated to test the presence of significance between the studied groups. Correlation between variables was performed using Pearson coefficient. P value \leq 0.05 was considered statistically significant [19].

2. Results.

2.1. Neurological and behavioral tests.

A significant difference in the number of adjusting steps of stepping test was observed between the studied groups. The number of adjusting steps was significantly lower in PD group than in C group. The treated groups (PD+Pir, PD+Vin and PD+Pir&Vin) showed significantly higher values, when compared with PD group, but were still significantly lower than that of C group. Combined piracetam and vincamine administration showed a significantly better improvement than their isolated administration (Fig 1-A).

The mean value of percentage of spontaneous alternation in Y-maze was significantly lower in PD group than in C group. The treated groups (PD+Pir, PD+Vin and PD+Pir&Vin) showed significantly higher values, when compared with PD group, but were still significantly lower than that of C group. Combined treatment in PD+Pir&Vin group showed significantly more percentage of spontaneous alteration than those of PD+Pir and PD+Vin groups; the latter groups showed nonsignificant variation from each other (Fig 1-B).

The duration of olfaction of the olfactory test was significantly lower in PD group than in C group. The treated groups (PD+Pir, PD+Vin and PD+Pir&Vin) showed significantly higher values, when compared with PD group, but were still significantly lower than that of C group (Fig 1-C). PD+Pir&Vin group showed a significantly more duration of olfaction than those of PD+Pir and PD+Vin groups; however, PD+Pir and PD+VIN groups were insignificantly different.

Significant differences in latency and immobility durations of swimming test were found among the studied groups. The latency time was significantly lower in PD group than in C group. The treated groups (PD+Pir, PD+Vin and PD+Pir&Vin) showed significantly higher values, when compared with PD group, but values were still significantly lower than that of C group. There was insignificant difference in latency time between PD+Pir and PD+Vin groups, but they were significantly lower than that of PD+Pir&Vin group. The immobility time was significantly higher in PD group than in C group. The treated groups (PD+Pir, PD+Vin and PD+Pir&Vin) showed significantly lower values, when compared with PD group, but values were still significantly higher than that of C group. PD+Pir and PD+Vin groups showed insignificant change in immobility time between each other. The immobility time of PD+Pir&Vin group was significantly less than those of PD+Pir and PD+Vin groups (Fig 1-D).

2.2. Biochemical analysis.

2.2.1. Serum biochemical measurements.

Table (1) demonstrates the various biochemical parameters measured in the serum of all the studied groups.

Fasting serum glucose level was significantly higher in the PD group than in C group. In PD+Pir, PD+Vin and PD+Pir&Vin groups, serum glucose levels were significantly lower than that of PD group, but they were still significantly higher than that of C group. Nonsignificant variations in serum glucose levels were observed between the three treated groups.

Serum calcium level was significantly lower in PD group than in C group. In PD+Pir, PD+Vin and PD+Pir&Vin groups, serum calcium levels were

significantly elevated as compared to PD group. Serum calcium in PD+Pir&Vin group was significantly higher than in PD+Vin group, but insignificantly changed as compared to PD+Pir group. The calcium levels in all treated groups were still significantly lower than that of C group. Serum CPK level was significantly higher in PD group than in C group. In PD+Pir, PD+Vin and PD+Pir&Vin groups, CPK levels were significantly lower than that of PD group. PD+Pir group showed significant decrease of serum CPK as compared to PD+Vin group. Serum CPK was significantly lower in PD+Pir&Vin group versus

PD+Pir and PD+Vin groups. Serum CPK of all treated groups were still significantly higher than that of C group.

Serum GDNF level was significantly lower in PD group than in C group. In PD+Pir, PD+Vin and PD+Pir&Vin groups, serum GDNF levels were significantly increased, when compared with PD group, but they were still significantly lower than that of C group. No significant variation was shown between PD+Pir and PD+Vin groups, however, PD+Pir&Vin group showed a significant increase in serum GDNF, when compared with both of them.

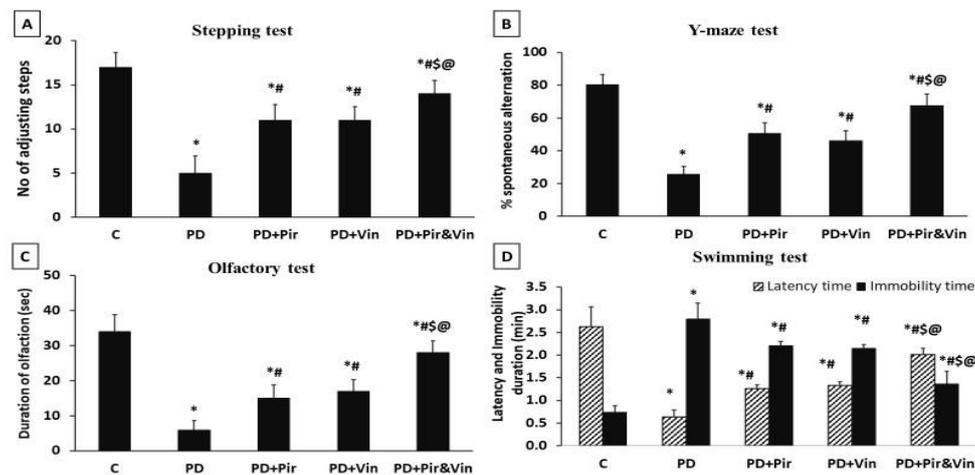


Figure (1): Neurological and behavioral test results in control (C), Parkinson's disease (PD), piracetam-treated Parkinson's disease (PD+Pir), vincamine-treated Parkinson's disease (PD+Vin) and piracetam- & vincamine-treated Parkinson's disease (PD+Pir&Vin) groups. Neurological and behavioral assessments were done as described in Materials and Methods. Results are expressed as mean \pm standard deviation (SD). Number of rats in each group was eight (n=8). Data were statistically analyzed by one-way ANOVA, and the level of significance was set on $P \leq 0.05$. *: significant versus PD+Pir group, @: significant versus PD+Vin group.

Table (1): Serum fasting glucose, calcium, creatine phosphokinase (CPK) and glial cell-derived neurotrophic factor (GDNF) levels in control (C), Parkinson's disease (PD), piracetam-treated Parkinson's disease (PD+Pir), vincamine-treated Parkinson's disease (PD+Vin) and piracetam- & vincamine-treated Parkinson's disease (PD+Pir&Vin) groups:

Group	C	PD	PD+Pir	PD+Vin	PD+Pir&Vin
Serum glucose (mg/dl)	87.82 \pm 4.68	191.42 \pm 4.66*	105.12 \pm 4.84*#	107.85 \pm 4.82*#	102.97 \pm 5.07*#
Serum calcium (mg/dl)	9.90 \pm 0.45	5.83 \pm 0.53*	8.77 \pm 0.58*#	8.60 \pm 0.51*#	8.93 \pm 0.59*#@
Serum CPK (U/ml)	124.27 \pm 7.5	411.5 \pm 11.2*	168.08 \pm 3.2*#	193.57 \pm 6.4*#	142.17 \pm 4.2*#S@
Serum GDNF (pg/ml)	129.87 \pm 6.57	50.83 \pm 8.19*	97.28 \pm 6.19*#	94.53 \pm 5.91*#	112.73 \pm 5.74*#S@

Biochemical parameters were measured as described in Materials and Methods. Results are expressed as mean \pm standard deviation (SD). Number of rats in each group was eight (n=8). Data were statistically analyzed by one-way ANOVA, and the level of significance was set on $P \leq 0.05$. *: significant versus C group, #: significant versus PD group, \$: significant versus PD+Pir group, @: significant versus PD+Vin group.

2.2.2. Striatal biochemical assays.

Table (2) demonstrates the various biochemical parameters measured in the supernatants of the striatal tissue homogenates of all the studied groups.

Regarding striatal homogenate dopamine level, there was a significant difference among the studied groups. Dopamine level was significantly lower in PD group than in C group. In PD+Pir, PD+Vin and PD+Pir&Vin groups, dopamine levels were significantly higher than in PD group, but they were still significantly lower than in C group. There was insignificant difference between dopamine levels in PD+Pir and PD+Vin groups, however, dopamine level in PD+Pir&Vin group was significantly higher than in any one of them.

Regarding oxidative stress markers, there were significant differences in striatal MDA and nitrite/nitrate levels among the studied groups. Striatal MDA and nitrite/nitrate levels in PD group were significantly higher than those in C group. Both MDA and nitrite/nitrate levels in PD+Pir, PD+Vin and PD+Pir&Vin groups were significantly reduced, when compared with those in PD group, but they were still significantly more than those in C group. The MDA level was significantly less in PD+Vin group than in PD+Pir group, while a nonsignificant variation in nitrite/nitrate level was observed between the two groups. Both striatal MDA and nitrite/nitrate levels in PD+Pir&Vin group were significantly decreased, when compared with corresponding values in PD+Pir and PD+Vin groups.

Regarding the antioxidants in striatal tissue homogenates, there were significant differences in the GSH level, and the GPx and SOD activities among the studied groups. The GSH level, and the

GPx and SOD activities in PD group were significantly less than those in C group. All the above-mentioned antioxidants were significantly elevated in PD+Pir, PD+Vin and PD+Pir&Vin groups, when compared with the corresponding values in PD group, but they were still significantly less than those in C group. PD+Vin group showed a significantly higher level of GSH, and activities of GPx and SOD than the corresponding values in PD+Pir group. PD+Pir&Vin group showed significant increase in GSH, GPx and SOD, when compared with those in PD+Pir group, but were insignificantly changed from those in PD+Vin group.

The striatal levels of the proinflammatory markers, TNF- α and IL-1 β , were significantly higher in PD group than in C group. In the treated groups (PD+Pir, PD+Vin and PD+Pir&Vin groups), both TNF- α and IL-1 β levels were significantly reduced, when compared with PD group, but they were still significantly more than those in C group. The TNF- α and IL-1 β levels were significantly less in PD+Pir group than in PD+Vin group, and both levels were significantly less in PD+Pir&Vin group than in PD+Pir and PD+Vin groups.

2.3. Correlation between Striatal dopamine level and other measured variables.

Table (3) shows the Pearson correlations of the dopamine level in the striatal tissue homogenate with the other studied variables.

Significant positive correlations were observed between the striatal dopamine level and the number of adjusting steps in stepping test, percentage of spontaneous alternations in Y-maze test, duration of olfaction in the olfactory test and latency time in swimming test for the control and

all the Parkinson's disease studied groups of rats in one analysis; a significant negative correlation of dopamine level with immobility time in swimming test was shown.

Also, there were significant positive correlations between the striatal dopamine level and the serum calcium, serum GDNF and striatal antioxidants (GSH level, GPx and SOD activities) of all studied groups in one analysis.

On the other hand, striatal dopamine level showed significant negative correlations with the serum glucose and CPK levels, oxidant markers, MDA and nitrite/nitrate level, and the striatal proinflammatory markers, TNF- α and IL-1 β , of all studied groups in one analysis.

Table (2): Dopamine, malondialdehyde (MDA), nitrite/nitrate, reduced glutathione (GSH), tumor necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β) levels, and the glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzymatic activities in striatal homogenates of control (C), Parkinson's disease (PD), piracetam-treated Parkinson's disease (PD+Pir), vincamine-treated Parkinson's disease (PD+Vin) and piracetam- & vincamine-treated Parkinson's disease (PD+Pir&Vin) groups:

Group	C	PD	PD+Pir	PD+Vin	PD+Pir&Vin
Dopamine (ng/g tissue)	0.40±0.07	0.06±0.05*	0.22±0.05*#	0.21±0.03*#	0.33±0.05*#@\$
MDA (nmol/g tissue)	2.29±0.28	12.22±0.64*	5.68±0.46*#	3.54±0.47*#	2.99±0.51*#@\$
Nitrite/nitrate (μ mol/g tissue)	11.67±5.5	31.33±6.06*	23.17±4.17*#	21.83±4.07*#	17.83±4.02*#@\$
GSH (mg/g tissue)	66.77±5.74	19.55±2.46*	31.6±5.18*#	55.72±6.17*#	56.90±6.48*#
GPx (U/g tissue)	32.07±0.93	7.49±0.57*	13.91±1.85*#	28.94±1.05*#	29.34±0.84*#
SOD (U/g tissue)	64.85±4.57	23.95±3.7*	34.42±5.76*#	54.59±6.62*#	54.37±5.49*#
TNF- α (ng/g tissue)	54.03±2.15	102.99±11.1*	66.79±3.87*#	80.56±3.52*#	60.16±3.67*#@\$
IL-1 β (pg/g tissue)	23.92±4.06	124.18±30*	40.95±6.74*#	72.08±5.91*#	32.28±5.97*#@\$

Biochemical parameters were measured as described in Materials and Methods. Results are expressed as mean \pm standard deviation (SD). Number of rats in each group was eight (n=8). Data were statistically analyzed by one-way ANOVA, and the level of significance was set on $P \leq 0.05$. *: significant versus C group, #: significant versus PD group, \$: significant versus PD+Pir group, @: significant versus PD+Vin group.

Table (3): Pearson correlation coefficient (r) between striatal dopamine level and the neurological and behavioral assessments for all rats (n=40):

	number of adjusting steps	Y-maze score (% of alternation)	olfactory test	swimming test	
				latency time	immobility time
Dopamine (r)	0.880	0.821	0.893	0.897	-0.909

Pearson correlation was significant ($P < 0.001$) for all parameters.

2.4. Histological examination.

The histopathological findings of SNpc in PD group showed decreased dopaminergic neurons and neuronal degeneration, inflammation, extensive hemorrhage and congested blood vessels, and numerous Lewis bodies, which were not seen in the normal structural appearance in control group. The PD+Pir, PD+Vin and PD+Pir&Vin groups showed improved histopathological picture of SNpc with an increase in dopaminergic neurons reflecting neuronal regeneration, and regression of inflammation indicating marked improvement compared to PD group (Fig 2).

Table (4): Pearson correlation coefficient (r) between striatal dopamine level and the measured biochemical variables for all rats (n=40):

	serum				striatal tissue homogenate						
	glucose	calcium	CPK	GDNF	MDA	nitrite/ nitrate	GSH	GPx	SOD	TNF- α	IL-1 β
Dopamine (r)	-0.774	0.843	-0.768	0.842	-0.760	-0.753	0.746	0.777	0.816	-0.781	-0.780

CPK: creatine phosphokinase, GDNF: glial derived neurotrophic factor, MDA: malondialdehyde, GSH: reduced glutathione, GPx: glutathione peroxidase, SOD: superoxide dismutase, TNF- α : tumor necrosis factor-alpha, IL-1 β : interleukin-1 beta. Pearson correlation was significant ($P < 0.001$) for all parameters.

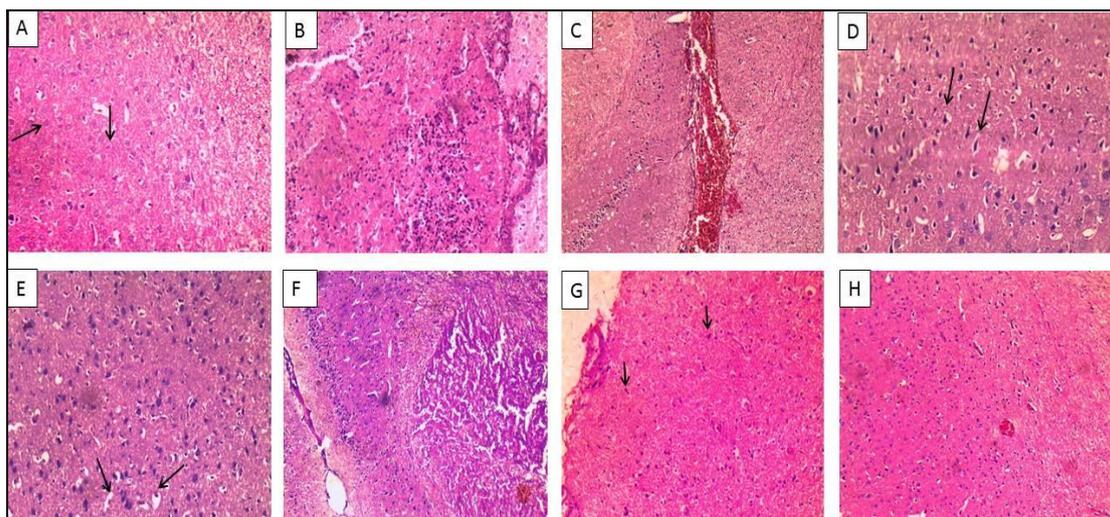


Figure (2): Photo micrographs of Hematoxylin and eosin (H&E)-stained sections of substantia nigra pars compacta (SNpc) ($\times 200$) from: (A) control group showing the normal histological picture of SNpc neurons with vesicular nuclei, prominent nucleoli and scant rim of basophilic cytoplasm. (B) Parkinson's disease group showing extensive lymphoplasmacytic inflammatory infiltration. (C) Parkinson's disease group showing extensive hemorrhage and congested blood vessels. (D) Parkinson's disease group showing neuronal degeneration in which neurons appeared with shrunken nuclei, inconspicuous nucleoli and surrounded with clear halo. (E) Parkinson's disease group showing numerous Lewis bodies (cytoplasmic eosinophilic inclusions). (F) piracetam-treated Parkinson's disease group showing gliosis and regression of inflammatory infiltrate. (G) vincamine-treated Parkinson's disease group showing mild vascular congestion and numerous regenerating neurons. (H) piracetam- and vincamine-treated Parkinson's disease group showing marked regression of both inflammation and congestion, with numerous regenerating neurons.

4. Discussion

Chronic haloperidol administration to rats for four weeks induced PD-like features [20] that was confirmed in the current study by the presence of bradykinesia, cognitive impairment, depressive-like behavior and hyposmia. The impaired neurological and behavioral tests in haloperidol-administered rats could be attributed to our observation of significant decrease in the striatal dopamine level, compared to normal animals; findings which are consistent with Amato et al. [21]. Loss of dopaminergic neurons in haloperidol-administered rats was demonstrated in this investigation by the

histopathological changes in substantia nigra, which is consistent with the observation of Abdelwahab et al. [22]. The impaired neurological and behavioral tests in PD rats were ameliorated in haloperidol-administered animals co-treated with piracetam or vincamine, and the combination of both agents exerted better improvement than treatment with each one alone.

Bradykinesia in PD rats was evidenced by decreased number of adjusting steps in stepping test, compared to normal animals; this could be attributed to a decrease of dopamine level in striatal tissue [23], as confirmed by presence of positive

correlation between striatal dopamine and stepping test. We observed a significant improvement in striatal dopamine in animals receiving piracetam or vincamine with haloperidol injection. In agreement, Zaitone et al. [24] have reported that vinpocetine, a synthetic derivative of vincamine, enhances the motor activity in a PD-rat model by restoring dopamine level in corpus striatum.

As dopamine is essential for working memory [25], we used Y-maze for its assessment. Our data showed that decreased dopamine level correlates the impairment of working memory with a reduction in percentage of spontaneous alternation; a finding that is in line with Handra et al. [26]. Treatment with either piracetam or vincamine improved the haloperidol-induced memory deficit. In agreement with the present results, Abdel-Salam and Nada [27] observed that piracetam alleviates the antipsychotic-induced impairment of learning and memory. Vincamine has been shown to improve speed of memory learning and recall in both cognitively healthy subjects and compromised subjects [28].

Rats with PD showed depressive-like behavior, as evidenced by increased immobility time of swimming test, when compared with normal animals. In fact, depression associates dopaminergic system dysfunction [29] that was indicated in the present study by the presence of negative correlation between striatal dopamine and immobility time of swimming test. An antidepressive effect was exerted by piracetam or vincamine, which is probably due to their neuroprotective effect, as evidenced by improvement in GDNF and increasing dopamine level in the striatal tissue. Piracetam is also a positive modulator of glutamate receptors and it

increases excitatory neurotransmission [30]. Vincamine is claimed to improve memory impairment accompanying neurodegenerative diseases by its antioxidant effect, increasing cerebral blood flow and glucose and oxygen utilization by the neurons [31].

We noted a positive correlation between dopamine level and duration of olfaction, reflecting role of dopamine in the olfactory process. Olfactory impairment in PD may be due to defective adult neurogenesis that can be modulated by dopamine [32]. Haloperidol-induced hyposmia was alleviated by treatment with piracetam, vincamine or both. The neuroprotective effect of piracetam could be explained by its ability to enhance membrane fluidity, especially that of mitochondria [33]. Restoring membrane fluidity promotes neurotransmission and enhances neuroplasticity [9]. We measured serum glucose level to study the effect of haloperidol and the used treated drugs on it, as observed from previous studies hyperglycemic effect of haloperidol [34], that could be corrected with piracetam and vincamine treatment [35, 36]. The findings of the current study revealed a significant increase of serum glucose in haloperidol-administered rats, as compared to normal ones. Chen et al. [34] had attributed the hyperglycemic effect of haloperidol due to induction of metabolic disturbances, insulin resistance, pancreatic cell dysfunction, dyslipidemia, and weight gain. Treatment with piracetam and vincamine improved the hyperglycemic state. In agreement with our results, Can et al. [35] reported improvements of hyperglycemia and cognitive dysfunction in diabetic patients upon treatment with piracetam, and explained their observation by an increase of

glucose uptake and oxidation in the neurons. Also, vincamine displays hypoglycemic effect by increasing cerebral blood flow and glucose utilization [36].

Disturbance of calcium homeostasis is supposed to be another key factor in the pathogenesis of PD [37]. A reduction of serum calcium level was observed in PD group, compared to normal rats. Co-administration of piracetam, vincamine or both with haloperidol was found to increase serum calcium. In fact, chronic administration of haloperidol encourages calcium influx via L-type calcium channels [38]. Both piracetam and vincamine act as calcium-channel blockers, so they reduce cellular calcium influx and preserve calcium in plasma [39].

CPK is an intracellular enzyme present in our body in heart, brain, and skeletal muscles, and other sites. It leaks into blood when muscle damage occurs, therefore high level of CPK denoting some sort of stress or injury affecting muscle leading to proteolysis and increasing membrane permeability [40], so, in this work we measured serum CPK level which found to be significantly increased after haloperidol administration, reflecting muscle damage [40], a finding which was in consistency with a previous study [12]. Serum CPK was reduced by co-administration of piracetam or vincamine with haloperidol, as both agents improve motor function and reduce haloperidol effect on skeletal muscles.

We measured serum GDNF as, it if found that it protects dopaminergic neurons in animal models of PD, and rescues motor neurons in vivo, raising hopes that it could be used as a therapeutic agent to treat several neurodegenerative diseases [41]. There was a decrease in serum GDNF in non-treated PD

rats, as compared to control animals. In support with our finding, Hisaoka et al. [42] proved that haloperidol decreases GDNF release from rat C6 glioma cells. In contrast, Amin et al. [12] reported an increase in GDNF level in haloperidol-administered rats. They explained their contradictory result by a possible up-regulation of GDNF after nigral neuronal injury. Co-administration of piracetam or vincamine with haloperidol resulted in an increase in serum GDNF. Oxidative stress is implicated in the pathogenesis of many neurodegenerative disorders, including PD [43]. Administration of haloperidol to rats resulted in an elevation of MDA, one of the major lipid peroxidation products, and total nitrite/nitrate levels, with concomitant reductions in the antioxidant GSH level and the GPx and SOD enzymatic activities in their striatal tissue, as compared to normal rats. These results agree with Zaidi et al. [44], who reported elevation of the oxidative stress and neuro-inflammatory markers in haloperidol-induced PD. Our data demonstrate a positive correlation of dopamine with antioxidants and a negative correlation with MDA and total nitrite/nitrate, denoting the role of oxidative stress in PD development and progression and the protective role of the antioxidant agents.

Haloperidol-induced oxidative stress was mitigated by treatment with piracetam or vincamine, as indicated by lower MDA and nitrite/nitrate and higher antioxidants in treated PD groups versus non-treated one, signifying an antioxidant function of both piracetam and vincamine that is in accordance with observations in other studies [28, 36]. In fact, both piracetam and vincamine preserve and protect dopaminergic neurons in a PD rat model by several mechanisms including antioxidant

and anti-inflammatory properties [24]. Piracetam enhances mitochondrial membrane fluidity by enhancing the function of the respiratory chain complexes [33]. Vincamine has an antioxidant scavenging capacity; it inactivates hydroxyl free radicals, in addition to its capability to prevent iron accumulation in rat brain [31].

Neuro-inflammation plays an important role in PD pathogenesis [45]. In consistency with previous studies [44, 46], haloperidol injection resulted in increases of the proinflammatory cytokines, TNF- α and IL1- β , in rat brain homogenate. Our data showed that co-administration of piracetam or vincamine to haloperidol-administered rats results in reduction of striatal levels of these proinflammatory cytokines, when compared with PD animals which did not receive any treatment, denoting an anti-inflammatory effect of both agents. Previous studies supported the anti-inflammatory effect of piracetam [24] and vincamine [47], which gives a new aspect about the role of nootropic drugs in the treatment of PD. However, Zaitone et al. [24] denies any significant effect of vinpocetine, the derivative of vincamine, on striatal TNF- α level. Interestingly, there was a significant negative correlation between the measured proinflammatory markers (TNF- α & IL1- β) and dopamine level in our work, indicating that they play a role in sustaining dopaminergic degeneration in PD, and denoting the role of neuro-inflammation in PD development and progression, and the protective role of the anti-inflammatory agents.

Neurodegeneration observed in histopathological finding in PD group may be due to inflammatory and oxidative stress state mediated by haloperidol administration that was mitigated by piracetam and

vincamine. Both agents improved histopathological changes in substantia nigra of PD rats, that was also seen by Zaitone et al. [24], possibly by increasing blood supply to the brain, delivering nutrient and energy required for neuronal function, improving neurotransmitter release, and having antioxidant and anti-inflammatory function [48].

The hallmark of our findings is that improvement of oxidative stress markers was more significant when treating PD rats with vincamine than piracetam, while reduction of pro-inflammatory cytokines was better with piracetam than vincamine. Striatal MDA level was lower, while the antioxidants GSH, GPx and SOD were higher in vincamine-treated PD rats than in piracetam-treated ones. The reduction of striatal TNF- α and IL1- β levels was more significant when treating PD rats with piracetam than vincamine. These results suggest that the neuroprotective effect of piracetam in haloperidol-induced PD arises more from its anti-inflammatory properties, whereas vincamine is more likely to achieve its protective effect via its antioxidant action.

The major finding of the present study is that the combined administration of both piracetam and vincamine to the haloperidol-induced PD rats ameliorated the associated deleterious effects better than administration of each one alone. Combined piracetam and vincamine administration to PD rats improved neurological and behavioral performance and serum calcium, CPK and GDNF levels better than in isolated piracetam or vincamine administration. Also, Striatal dopamine, MDA, nitrite/nitrate, TNF- α and IL1- β levels showed better improvement in combined treatment than in case of treatment with either piracetam or vincamine alone. The better amelioration of the

neurological and biochemical errors by the combined administration of piracetam and vincamine could be explained by an additive effect, as the two agents belong to distinct chemical classes and have different mechanisms of action. However, potential adverse effects of using the two agents in combination may exist, and should be investigated to ensure that their combination has a beneficial effect in protection of the detrimental effects of PD.

Limitations to this study, doses of used nootropic drugs, that we recommend further studies to assess the effect of different doses of these drugs. Also, more techniques like immunohistochemistry and PCR may be more helpful in such work.

In conclusion, both piracetam and vincamine exhibit neuroprotective effect in haloperidol-induced PD rat model and modulate striatal dopamine release, and thus could be considered as possible candidates for management of PD. The combination of both nootropic agents affords a better protection than the use of each one alone that could be explained by an additive effect based on their different mechanisms of action. Piracetam reaches its effect mainly by its anti-inflammatory action, while vincamine has mainly antioxidant properties. It is recommended to study these agents thoroughly in clinical trials for use in PD, and to investigate possible adverse effects of their combination, if any.

5. Conflict of interest:

The authors declare that there is no conflict of interest associated with this work.

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