Neuroprotective Effects of Piracetam Versus Peroxisome Proliferator-Activated Receptor-Gamma Agonist Pioglitazone in Drug-Induced Parkinsonism

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ABSTRACT

Parkinsonism is a common and disabling neurodegenerative disease. Drug-induced Parkinsonism is not uncommon. The neuroprotective actions of Piracetam and peroxisome proliferator–activated receptor gamma (PPAR γ) agonist pioglitazone have been reported. The aim of the current work was to investigate and compare the neuroprotective effect of piracetam versus pioglitazone on haloperidol-induced Parkinsonism. 36 male rats constituted the animal model for our study and had been divided into 6 groups (6 rats/group); control, Haloperidol, Piracetam, Piracetam with haloperidol, Pioglitazone, and Pioglitazone with Haloperidol groups. Hypokinesia was tested by stepping test and blood samples were collected for measurement of serum glucose, calcium, Creatine phosphokinase (CPK), Transforming growth factor beta 1 (TGF-β1), Fibroblast growth factor 21 (FGF-21), Glial cell-Derived Neurotrophic factor (GDNF). Brains extracted for measurement of basal ganglia Tyrosin hydroxylase (TH) and Beclin gene expression. Haloperidol caused hypokinesia, increased serum glucose, CPK, TGF-β1, FGF-21, GDNF, basal ganglia TH expression and decreased serum calcium and basal ganglia Beclin expression. Both piracetam and pioglitazone provided neuroprotection and improved hypokinesia and the biochemical markers measured in blood and brains of haloperidol-induced Parkinsonism when administered before haloperidol, however; Pioglitazone had a better effect regarding the improvement of serum glucose, CPK and brain expression of Beclin.

Keywords: Parkinsonism, Haloperidol, Piracetam, Pioglitazone, Neuroprotection

1. INTRODUCTION

Parkinsonism is a clinical syndrome characterized by stiff muscles, slow movements, tremor and often postural instability. There are various conditions that can cause Parkinsonism such as environmental(1) or heritable causes(2). Parkinsonism can be induced through drug-related(3), of those drug-inducing Parkinsonism, the dopamine inverse agonist, Haloperidol. It causes dopaminergic D2 and serotonergic 5-HT2A receptors blockade(4) and it is used as an antipsychotic in the treatment of delirium, acute psychotic states, and schizophrenia(3). Piracetam, a nootropic drug and used in the treatment of cognitive impairment(5), cerebral stroke(6) and dementia in the elderly(7). Piracetam was found to induce neuroprotective activity(8).
Pioglitazone is an agonist for peroxisome proliferator-activated receptor gamma (PPARγ). Pioglitazone decreases insulin resistance. Pioglitazone showed neuroprotection in Alzheimer’s disease, stroke and epilepsy. The aim of this study was to evaluate and compare the neuroprotective effects of piracetam versus pioglitazone in an animal model of haloperidol-induced Parkinsonism.

2. METHODS

Study design and experimental groups
The experimental procedures, animal handling, sampling, and scarification were performed according to the Guide for the care and use of laboratory animals, Eighth Edition. 36 Male albino rats aged 2-3 months, with weight ranging from 120 to 150 gm constituted the animal model for this study. Rats were housed 3 per cage at a constant temperature (22-24 °C) and light controlled room on an alternating 12:12 h light-dark cycle with free access to food and water and were fed a standard commercial pellet diet.

Rats were divided into the following groups (6 rats/group):

1. **Group I (control)**: received daily intraperitoneal injection of 0.9% saline.
2. **Group II (Haloperidol group)** received haloperidol (5 mg/kg) daily for 4 weeks provided as commercial ampules (Haloperidol ampules, Nile Company for Pharmaceutical, 5mg/ml).
3. **Group III (Piracetam group)** received piracetam (300 mg/kg, i.p) provided as commercial ampules (Cerebrocetam ampules, PHARCO Pharmaceuticals – Alexandria), daily for 4 weeks.
4. **Group IV (Piracetam +Haloperidol group)** received piracetam (300 mg/kg, i.p) and after 30 minutes received haloperidol (5 mg/kg i.p) daily for 4 weeks.
5. **Group V (Pioglitazone Group)** received Pioglitazone (10 mg/kg, p.o.) as a suspension in sterile water by oral gavage.
6. **Group VI (Pioglitazone + Haloperidol)** received Pioglitazone as a suspension in sterile water at a dose of (10 mg/kg, p.o.) by oral gavage and after 30 minutes received haloperidol (5 mg/kg i.p) daily, for 4 weeks.

Assessment of hypokinesia-Stepping test
This test was performed twice; at the beginning of the study and just before scarification. We firmly suspended the rat’s hindquarters while it supported its weight on its forelimbs. Then, the rat moved backward along the table (0.9 m in 5 seconds) three times consecutively per session. For each session; the total score calculated was the sum of the number of adjusting steps observed in the three tests.

Biochemical measurements
At the end of the experiment, rats were anesthetized, blood is withdrawn from retroorbital venous sinuses for serum analysis of glucose, calcium, Creatine phosphokinase (CPK), Transforming growth factor beta 1 (TGF-β1), Fibroblast growth factor 21 (FGF-21), and Glial cell-Derived Neurotrophic Factor (GDNF). Rats were sacrificed, brain extracted and dissected at the level of the basal ganglia for evaluation of Tyrosine hydroxylase and Beclin gene expression.

Serum calcium and glucose were measured by calorimetric methods according to manufacturer’s instruction (Abcam, USA). Serum CPK Kit is based on enzyme coupled reactions according to manufacturer’s instructions (Abnova, Taiwan).

Levels of TGF-β1, FGF-21, and GDNF were determined by using a corresponding ELISA kit purchased from BD Biosciences (San Jose, CA) according to the protocol provided by the manufacturer.

Beclin and tyrosine hydroxylase gene expressions were detected using (qRT-PCR).

Statistical analysis
Data were coded and entered using the statistical package SPSS version 21 (IBM SPSS Statistics 21; IBM Corporation, New York, USA) for Microsoft Windows. Data was summarized using mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc Benferoni test. P-values less than 0.05 were considered as statistically significant.

3. RESULTS

Assessment of the studied groups after 4 weeks of drug administration revealed the following results as demonstrated in figure (1) and table (1).
Fig. (1): Sum of the number of adjusting steps in stepping test in the studied groups.

Table (1): Biochemical Parameters measured in serum and brains of the studied groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I Control Group (n=6)</th>
<th>Group II Haloperidol Group (n=6)</th>
<th>Group III Piracetam Group (n=6)</th>
<th>Group IV Piracetam-Haloperidol (n=6)</th>
<th>Group V Pioglitazone (n=6)</th>
<th>Group IV Pioglitazone-Haloperidol (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Glucose (mg/dl)</td>
<td>82.23±4.54</td>
<td>181.8±18.04 *</td>
<td>87±5.8</td>
<td>103.35±4.63 * b</td>
<td>104±7.31 a</td>
<td>94.7±4.05 b</td>
</tr>
<tr>
<td>Serum CPK (u/L)</td>
<td>126.9±2.3</td>
<td>205.98±13.77 *</td>
<td>139.55±4.45 b</td>
<td>143.1±7.2216 * a b</td>
<td>131.65±3.58 a</td>
<td>139.8±11.12 b</td>
</tr>
<tr>
<td>Serum Calcium (mg/dl)</td>
<td>10.12±.397</td>
<td>7.78±1.06 * a</td>
<td>9.14±.62</td>
<td>10.41±.41 b</td>
<td>9.88±.37 c</td>
<td>8.53±.87 abc</td>
</tr>
<tr>
<td>Serum TGF-β1 (pg/ml)</td>
<td>37.3±4.48</td>
<td>295.63±22.9 * b</td>
<td>44.26±11.26</td>
<td>88.48±11.09 * b a</td>
<td>40.38±5.23</td>
<td>85.1±6.9 ab</td>
</tr>
<tr>
<td>Serum FGF-21 (pg/ml)</td>
<td>44±3.6</td>
<td>192.8±24.8 * b</td>
<td>66.7±14.59</td>
<td>105.2±8.42 a b</td>
<td>61.38±12.47</td>
<td>104.2±22.58 b</td>
</tr>
<tr>
<td>Serum GDNF (ng/ml)</td>
<td>.53±.186</td>
<td>2.340±1.66 * b</td>
<td>.9083±14.289</td>
<td>1.36±.15 a b</td>
<td>.91±.17</td>
<td>1.96±.3 ab</td>
</tr>
<tr>
<td>Basal ganglia TH</td>
<td>1.096 ±0.82</td>
<td>11.32±1.65 * a</td>
<td>1.591±.61</td>
<td>5.37±1.59 a b</td>
<td>1.19±.255</td>
<td>3.56±.45 ab</td>
</tr>
<tr>
<td>Basal Ganglia Beclin</td>
<td>1.23±.311</td>
<td>.2±.048 a</td>
<td>.99±.17</td>
<td>.57±.17 a b</td>
<td>1.07±.166</td>
<td>.73±.17 ab</td>
</tr>
</tbody>
</table>

CPK: Creatine Phosphokinase, TGF-β1: Transforming Growth Factor Beta 1, FGF-21: Fibroblast Growth Factor 21, GDNF: Glial Cell Derived Neurotrophic Factor, TH: Tyrosine Hydroxylase. a Compared to control group; b Compared to haloperidol group; c Compared to Piracetam +Haloperidol group at P value ≤0.05

When the rats tested by stepping test, they showed significant (P≤0.05) decrease of the number of adjusting steps in Haloperidol group compared to control group. Pretreatment with piracetam or pioglitazone significantly improved hypokinesia and resulted in a significant (P≤0.05) increase of the number of adjusting steps compared to Haloperidol group, however; they did not normalize it to control level.

There was a significant (P≤0.05) increase of serum glucose and CPK in Haloperidol group compared to control group. Piracetam administration before haloperidol significantly (P≤0.05) decreased serum glucose and CPK compared to Haloperidol group, but levels still significantly increased compared to control group. On the other hand, Pioglitazone therapy before daily haloperidol significantly (P≤0.05) decreased serum glucose, and CPK and these levels were insignificantly different from those in the control group.

Serum calcium was significantly (P≤0.05) decreased in Haloperidol group compared to control group. Piracetam therapy before daily haloperidol improved serum level of calcium and significantly (P≤0.05) increased it compared to Haloperidol group, and it almost normalized it to control value. However, pioglitazone administration before haloperidol did not improve serum calcium level compared to Haloperidol group and level was significantly (P≤0.05) decreased compared to control group.

A significant (P≤0.05) increase of serum TNF-β1, TGF-21 and melatonin in the group treated with haloperidol compared to control group. Administration of piracetam or pioglitazone before the daily haloperidol dose resulted in significant (P≤0.05) decreases of serum TGF-β1, TGF-21 and melatonin compared to Haloperidol group. However, the levels were significantly (P≤0.05) increased compared to control group.

Serum GDNF was significantly (P≤0.05) decreased in Haloperidol group. Therapy with piracetam or pioglitazone before haloperidol caused significant (P≤0.05) increase compared to Haloperidol group. However, both drugs did not restore the level of GDNF to control value.

Basal ganglia tyrosine hydroxylase gene expression was significantly (P≤0.05) decreased in Haloperidol group compared to control group. Both piracetam and pioglitazone increased tyrosine hydroxylase expression in the basal ganglia when given before daily haloperidol dose. However, expression remained significantly (P≤0.05) decreased compared to control group.

There was a significant (P≤0.05) decrease of Basal Ganglia expression of Beclin in Haloperidol group. Administration of either piracetam or pioglitazone before haloperidol significantly (P≤0.05) increased Beclin expression. However, both drugs did not restore the Beclin expression to control value.

4. DISCUSSION

Drug-induced Parkinsonism is the most common movement disorder induced by drugs\(^3\). We assessed and compared the effects of pioglitazone versus piracetam on biochemical markers in serum and brains of haloperidol-induced Parkinsonism. Haloperidol blocks post-synaptic D2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects\(^20\).

In the present work, the hypokinesia induced by haloperidol improved in groups pretreated by piracetam or pioglitazone and almost both drugs exerted a similar degree of improvement of hypokinesia. In agreement with our finding; a study by Abdel-Salam and Nada\(^16\), showed that piracetam improved akinesia induced by haloperidol. Piracetam restores the number of active GABA-A receptors in rats made anxiolytic and depressed by prolonged hypokinesia, increases blood flow, affects membrane fluidity and glucose transport into the cells\(^16\).

Pioglitazone showed neuroprotective effects by decreasing microglial activation, mitochondrial dysfunction, and expression of various inflammatory mediators\(^21\).

We observed a significant increase in serum glucose in Haloperidol and Pioglitazone groups compared to control. Treatment with either pioglitazone or piracetam before haloperidol resulted in significant decrease in serum glucose compared to group injected with haloperidol alone with better euglyemic effect with pioglitazone.

Our results agree with previous studies, on patients treated with atypical antipsychotics drugs like haloperidol and showed that they are more likely to develop diabetes and hyperglycemia\(^22\). Hyperglycemia could be related to the hyperprolactinema caused by the typical antipsychotics drugs as causing an increased insulin resistance\(^23\). Haloperidol antagonizes dopamine D2 receptors, and the activation of these receptors enhances insulin secretion\(^24\). Activation of dopamine
D2 receptors ameliorates insulin resistance in obese women through a mechanism that is independent of body weight\(^{(25)}\).

In agreement with our results; Pandey and Garabadu\(^{(26)}\), reported a hypoglycemic effect of piracetam in type-2 diabetic encephalopathic rats. Pioglitazone binds to PPAR\(\gamma\) receptor, promotes glucose uptake by increasing expression of insulin receptor substrate-2 and glucose transporter 4 in insulin-sensitive tissues\(^{(27)}\). The hyperglycemic effect of pioglitazone in the positive control group may be explained by the suppressive effect of pioglitazone on insulin secretion by beta cells which is PPAR\(\gamma\) independent\(^{(28)}\).

Our results showed that haloperidol significantly decreased Serum Calcium level compared to control and administration of piracetam before haloperidol significantly increased serum calcium level compared to Haloperidol group and almost normalized it. Pioglitazone treatment before Haloperidol showed an increase in Serum Calcium level compared to Haloperidol group alone but still below the normal level. In contrast to our results; Solntseva et al.\(^{(29)}\), reported that Piracetam reduced cytoplasmic calcium levels in addition to blocking calcium ion channels. Pioglitazone increases proximal sodium reabsorption; it might have an effect on proximal calcium reabsorption thus an increase of calcium excretion rate as proposed by Zanchi et al.\(^{(30)}\). That agrees with another study by Zanchi et al.\(^{(31)}\), in which pioglitazone was found to increase urinary calcium excretion up to 45% because it decreased the alkaline phosphatase significantly.

Plasma CPK is a marker signifying muscle injury arising from myofibrillar disruption\(^{(32)}\). Our results showed that there was a significant increase in serum CPK in Haloperidol group compared to control. Treatment with either piracetam or pioglitazone before haloperidol injection resulted in significant decrease in serum CPK compared to Haloperidol group. Meltzer et al.\(^{(33)}\), showed massive skeletal muscle damage as serum CPK was monitored in schizophrenic patients treated with antipsychotics like haloperidol. In contrast to our findings; Pioglitazone was shown to have an adverse effect of elevating CPK levels accompanied by proximal muscle weakness\(^{(34)}\).

TGF-\(\beta\) is an immunomodulatory cytokine, and its signaling is involved in the control of T-cell activation and inflammation\(^{(35)}\). We observed a significant increase in serum TGF-\(\beta\)1 in Haloperidol group compared to the control group and significant decrease in serum TGF-\(\beta\)1 levels in both groups that received piracetam or pioglitazone before Haloperidol.

Cohen et al.\(^{(36)}\) showed that antipsychotics caused an increase phosphorylation of SMAD3, which is a downstream effector of TGF\(\beta\) pathway. Piracetam may affect serum TGF-\(\beta\)1 indirectly through the serum calcium levels as it was proven that Piracetam decreases cytoplasmic calcium levels\(^{(29)}\) that have a strong correlation with TGF-\(\beta\) mediated stimulation\(^{(37)}\).

In contrast to our finding; Wang et al.\(^{(38)}\) showed that pioglitazone had potential therapeutic protection from ischemia in myocardium via upregulation of TGF-\(\beta\)1. However, it can cause TGF-\(\beta\)1 mediated suppression at higher concentrations in vitro on endothelial progenitor cells as demonstrated by Redondo et al.\(^{(39)}\). The TGF\(\beta\) pathway and SMAD3 are associated with insulin resistance supporting that type II diabetic patients have higher serum TGF\(\beta\) than normal controls\(^{(40)}\).

FGF-21 is a metabolic regulator shown to facilitate glucose and lipid metabolism\(^{(41)}\). FGF-21 has also been implicated to have a protective role in dopaminergic neuron degeneration by promoting cell survival and neurogenesis\(^{(42)}\). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1\(\alpha\)) is a master regulator of mitochondrial function by regulating the gene expression of mitochondrial genes\(^{(43)}\). FGF21 significantly increases the expression level of PGC-1\(\alpha\). Activation of PGC-1\(\alpha\) affects the expression of a variety of different genes including mitochondrial antioxidants\(^{(44)}\).

We reported a significant increase in serum FGF-21 in Haloperidol group compared to control, while groups treated with either piracetam or pioglitazone before haloperidol, noticeably decreased Serum FGF-21 levels in both compared to Haloperidol group alone. FGF-21 is upregulated in adipose tissue through PPAR\(\gamma\) and controls PPAR\(\gamma\) activity in an autocrine manner to enhance adipogenesis and to mediate the clinical and side effects of PPAR\(\gamma\) agonist thiazolidinedione\(^{(45)}\).

In contrast to the haloperidol elevated serum FGF-21 levels, Riva et al.\(^{(46)}\) showed that haloperidol did not increase FGFs levels.

GDNF is a neurotrophic factor for dopaminergic neurons\(^{(47)}\). The current work revealed an increase of serum GDNF in the haloperidol-treated group compared to control. Pre-treatment of Haloperidol group with either piracetam or pioglitazone resulted in significant decrease in the GDNF compared to the
group treated with haloperidol alone. In agreement with our results; Shao et al.\(^\text{48}\) proved that haloperidol increased GDNF secretion and release in glioblastoma cells. However; Hisaoka et al.\(^\text{49}\) demonstrated that treatment with non-antidepressant psychotropic drugs in rats did not elevate GDNF in glioblastoma cells. In substantia nigra neurons, tyrosine hydroxylase (TH) catalyzes the synthesis of L-3,4-dihydroxyphenylalanine (L-DOPA) from tyrosine, which is the rate-limiting step in DA biosynthesis. Thus, TH plays a crucial role in PD pathogenesis\(^\text{50}\).

We reported a significant increase in Basal ganglia TH in the Haloperidol-treated group compared to the control one. Treatment with either pioglitazone or piracetam before haloperidol significantly decreased Basal ganglia Tyrosine hydroxylase level compared to Haloperidol group; however, treatment with pioglitazone before haloperidol seems to be more significant than piracetam. Meloni and Gale\(^\text{51}\) showed that haloperidol administration caused an activation of tyrosine hydroxylase in the transplant terminals with an increase of its affinity to pteridine cofactor; supporting our results of the increased tyrosine hydroxylase levels in Haloperidol group.

Our results showed that Haloperidol decreased Basal Ganglia Beclin levels significantly compared to the control group. Treatment with piracetam before haloperidol showed an increase in Basal Ganglia Beclin levels compared to Haloperidol alone. However, pioglitazone treatment before haloperidol seems to increase Basal Ganglia Beclin levels more than piracetam. Beclin is one of the key regulators of autophagy and a substrate for AKT-dependent phosphorylation\(^\text{52}\).

Chronic treatment of mice with Haloperidol increased GSK-3β at Ser-9 thus increasing AKT phosphorylation\(^\text{53}\), not only in the striatum but also the prefrontal cortex and ventral midbrain\(^\text{54}\). This phosphorylation of GSK-3β is able to reduce its kinase activity and offers cell survival in autophagy\(^\text{55}\).

In their study, Xu et al., \(56\) found that natural PPARγ ligand possesses a protective role in ischemia-reperfusion especially 15-deoxy-delta12, 14-prostaglandin J2 (15-PGJ2) as it decreased the expression of autphagic proteins including Beclin\(^\text{56,57}\).

### 5. CONCLUSION

We conclude that both piracetam and pioglitazone improved hypokinesia and the biochemical markers measured in blood and brains of haloperidol-induced Parkinsonism. Pioglitazone was better than piracetam regarding the improvement of serum glucose, CPK and brain expression of Beclin. Further studies are recommended to investigate the possible mechanism by which pioglitazone induced hyperglycemia in the positive control group and hypoglycemia when administered with haloperidol using the same dose and duration of therapy. Further cognitive, behavioral and olfactory assessment for the given drugs is recommended.

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CONFLICT OF INTEREST

There is no conflict of interest.

### REFERENCES


