Research Article

Evaluation of Anti-parkinson's Activity of Novel Glitazones on Rats

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ABSTRACT

Parkinson's disease is currently considered as the second commonest neurodegenerative disorder in the world. The present study was undertaken to investigate the anti-Parkinson's activity of novel glitazones derivatives (C10 and C12) on experimental rat model of in vivo haloperidol induced Parkinson's disease (PD). Before in-vivo activity docking studies of novel glitazone was performed against docking protein 3CS8 using Sybil software. Acute toxicity study of novel compounds was performed by OECD guideline 423. The compounds were screened at two dose levels of 10 and 20 mg/kg for 7days. On 7th day behavioural parameters like muscle coordination by rota rod apparatus, locomotor activity by actophotometer and muscle strength by staircase method were evaluated and brains were used for estimation of antioxidant enzyme activity. The compounds were safer at 300 mg/kg body weight when tested by OECD guideline 423. Out of five novel compounds two compounds C10 and C12 were selected based on the docking study results, in docking study these two compounds were found to show better receptor protein binding activity. At dose of 20mg/kg compound C10 showed significant anti-Parkinson's activity when compared to C12. Hence, we conclude that these novel glitazone compounds have some anti-Parkinson's activity. But further studies are required to support the present assumption and elucidate detailed neuroprotective mechanism.

Keywords: Glitazone, Anti-Parkinson's, Haloperidol, levodopa, OECD.

INTRODUCTION

Dr. James Parkinson was the first scientist described the Parkinson's disease (PD) as a "shaking palsy" in the year 1817[1]. It is a long-lasting, progressive disorder of the CNS and the disease mainly affects the motor functions. Loss of dopamine causes neurons to fire without normal control, leaving patients less able to direct or control their movement[2]. The recent evidences suggested that the co-activator of PPARγ that is PGC-1α plays an important role in the regulation of mitochondrial biogenesis. The expression of PGC-1α is reduced in the substantia nigra of PD patients. It also showed the protective mechanism towards the anti-inflammatory and anti-oxidative effects, but the specific mechanism is unclear. The main hypothesis of the present study is glitazones helps in activation of expression of PGC-1α and provides the neuroprotection effects through the regulation of mitochondrial function[3].

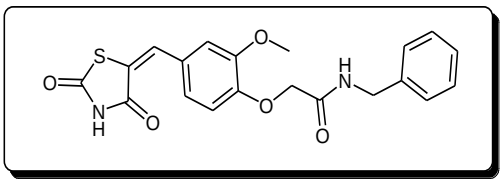
The present study was conceived with objectives of evaluation of anti-Parkinson's activity of two newly derived compounds of glitazones (C10 and C12) against haloperidol rat model. Docking studies was also performed to know the extent of binding of novel glitazone for the target protein.

MATERIALS AND METHODS

The experiment was carried out on male Wister Albino rats weighing between 200-250 gm, the animal care and handling was carried out in accordance with CPCSEA guidelines issued by the Institutional Animal Ethics Committee, JSS College of Pharmacy, Mysore, Karnataka. The two newly derived glitazones were synthesised and were named as C10 and C12 as reference code and were used for the study. The compounds were weighed and stored in an air-tight container at room temperature.

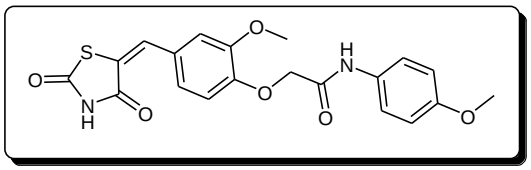
Structure elucidation of compounds

Compound 10



N-Benzyl-2-{4-[thiazolidine-2,4-dion-(5Z)-ylidenemethyl]-2-methoxy-phenoxy}-ethanamide

Compound 12



2-{4-[Thiazolidine-2,4-dion-(5Z)-ylidenemethyl]-2-methoxy-phenoxy}-N-(4-methoxy-phenyl)-ethanamide

Molecular docking study

In the present study we have selected the target protein as 3CS8 with which the ligands are interacted and finally the Docking poses were visualized. The main Docking

operation was performed on "DOCK LIGAND" wizard, under SYBYL X 2.1.1 SOFTWARE Package, where the prepared proteins and prepared ligands were taken for further study. The active site of the protein was identified by "PROTOMOL GENERATION" Wizard and the ligands were docked along with the co-crystallized ligand which was present within the crystal structure of protein molecule, finally the binding poses were observed and docking score was obtained (TOTAL SCORE, CRASH SCORE, POLAR SCORE) by using SYBYL X 2.1.1 scoring function.

Acute Toxicity study

Acute toxicity study was performed based on OECD guideline 423 for two compounds C10 and C12.

In-vivo activity

This study was done for a period of 7 days where on 7th day behavioural parameters were assessed and brains were isolated and estimated for anti-oxidant enzyme activity. The rats were divided in to 7 groups of 6 animals per group and treatments were given as shown in Table1.

Table 1: Grouping, treatment and evaluation

Groups	Treatment	Evaluation parameters
Normal	0.5% Sod CMC given (1ml/kg BW, per oral)	**Behavioral parameters**
Control	Vehicle (1ml/kg BW per po) +Haloperidol (1mg/kg BW, i.p.) every day after 1 hr of regular treatment.	Rota rod[4] Actophotometer[5] Stair case method[6]
Standard	L-dopa-carbidopa (110/10kg BW, p.o) + haloperidol (1mg/kg BW, i.p.) every day after 1 hr of regular treatment.	**Antioxidant enzymes** SOD[7] LPO[8] GSH[9]
C10D1+ Haloperidol	(10mg/kg BW) prepared in 0.5% sodium CMC p.o + haloperidol (1 mg/kg BW, i.p.) every day after 1 hr of regular treatment.	
C10D2+Haloperidol	(20mg/kg BW p.o) + haloperidol (1 mg/kg BW, i.p.) every day after 1 hr of regular treatment.	
C12D1 +Haloperidol	(10mg/kg BW p.o) + haloperidol (1 mg/kg BW, i.p.) every day after 1 hr of regular treatment.	
C12D2+Haloperidol	(20mg/kg BW p.o) + haloperidol (1 mg/kg BW, i.p.) every day after 1 hr of regular treatment.	

Data analysis

Data were expressed as mean ± standard error of the mean (SEM). Mean difference between groups were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison test using GraphPad Prism 5.0 (San Diego, USA) software. P value ≤ 0.05 was considered as significant.

RESULTS

Molecular Docking study

The docking studies reveal that the binding ability of novel compound C10 showed better interaction with PPAR receptors than compound C12 when compared with the reference ligand (rosiglitazone). The standard levodopa has showed the less binding activity when compared with reference ligand because of its less binding activity with the target protein 3CS8 (Table 2). This reveals that the PPAR receptors showed the hypothesis through binding to PGC-1α which helps in the improvement of mitochondrial biogenesis which further helps in neuroprotection activity.

Table 2: The Score obtained after Molecular docking process

Name	Total score	Crash	Polar
C10	8.2866	-2.4825	1.406
C12	7.5037	-2.4825	1.8487
Reference Ligand	7.2022	-1.324	1.9837
Levo dopa	4.7553	-2.0953	1.6198

Acute oral toxicity

The acute toxicity of 2 compounds, C10 and C12 were tested for 14 days. Where at the dose of 300 mg/kg it did not showed any clinical sings of toxicity. Based on the safety acute toxicity study we used 10 and 20 mg/kg of newly derived glitazone compounds for *in-vivo* studies.

Behavioural Parameters

Muscular coordination activity

Table 3: Anti-parkinsonism activity of novel glitazones derivatives on haloperidol induced Parkinson disease in rat model (Muscle coordination)

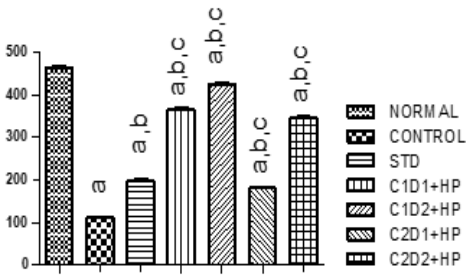
Groups	Muscle coordination by rota rod test
Normal	206.33±2.28
Control	100.50±2.81[a]
Standard	161.83±4.86[a, b]
C_{10} D1+HP	151.83±3.08[a, b]
C_{10} D2+HP	193.50±2.48[b, c]
C_{12} D1+HP	150.11±4.51[a, b]
C_{12} D2+HP	186.13±1.94[a, b, c]

All values are expressed as Mean ± SEM n=6. *Statistical analysis was analysed by One-way ANOVA followed by Tukey's multiple comparison test [a]P<0.05 compared to*

normal, [b]P<0.05 compared to control [c]p<0.05 compared to standard.

The animals were placed on the rotarod and absorbed for the latency of fall. The latency of fall off time was more in the normal group (206.33±2.28) when compared with the control group hence it showed the significant induction of the PD. When we compared the test compound with the control group (100.50±2.28), the test compounds showed the significance muscle coordination & gripping action (151.83±3.06), (193.50±2.48), (186.13±1.943) and (150.11±4.51). The test compound C10 and C12 showed the better gripping action at the dose of 20mg/kg when compared with the standard (161.83±4.86). The test compound C1O (193.50±2.48) has showed the better action when compared with the compound C12 (186.13±1.94).

Spontaneous locomotory activity (SLA)



the values are expressed as MEAN±SEM n=6
the motor activity was analysed by one way ANOVA followed by Tukey's multiple comparision test
[a]P<0.05 compared to normal,[b]P<0.05 compared to control,[c]P<0.05 compared to standard

Figure 1: Anti-parkinsonism activity of novel glitazones derivatives on haloperidol induced Parkinson activity in mice (locomotory activity).

The motor activity was less in the control group when compared with the normal group (463.00±2.91) hence it showed the significant induction of the PD. When we compared the test compound with the control group (111.83±2.57), the test compounds showed the significance motor activity (364.16±3.93), (425.00±3.26), (180.00±2.59) and (344.00±3.93). The test compound C10 and C12 showed the better motor activity at the dose of 20mg/kg when compared with the standard (198.83±2.61). The test compound C1O at dose of 20mg/kg (425.00±3.26) has showed the better action when compared with the compound C12 (344.00±3.93).

Stair case activity

The muscle strength was analysed by One-way ANOVA followed by Tukey's multiple comparison tests [a]P<0.05 compared to normal [b]P<0.05 compared to control [c]p<0.05 compared to standard

Mainly this study was done to evaluate the muscle activity of mouse. The cut off time employed in this method was 3 minutes. Here mainly two compounds were used to test the anti-Parkinson's activity. The time taken to climb the steps by control group animals was more when compared with the normal group (27.00±1.23) hence it showed the significant induction of the PD. When we compared the test compound with the control group (131.83±1.57), the test compounds showed the significance motor activity (58.83±2.03), (52.33±1.90), (75.00±1.58) and (72.50±1.56). The test compound C10 and C12 showed the less motor activity when compared with the standard (47.83±1.32). The test compound C1O has showed the better action when compared with the compound C12.

Table 4: Anti-parkinsonism activity of novel glitazones derivatives on haloperidol induced Parkinson activity in mice (Muscle activity)

Groups	Muscle activity by staircase (Seconds)
Normal	27.00±1.23
Control	131.83±1.57[a]
Standard	47.83±1.32[a, b]
C_{10} D1+HP	58.83±2.03[a, b, c]
C_{10} D2+HP	52.33±1.90[a, b]
C_{12} D1+HP	75.00±1.58[a, b, c]
C_{12} D2+HP	72.50±1.56[a, b, c]

All values are expressed as Mean ± SEM n=6

Biochemical Parameters

Evaluation of brain endogenous antioxidant enzymes:

The biochemical parameters were estimated in brain by estimating the antioxidant enzymes which plays a major role in the parkinson disease.

Table 5: Anti-parkinsonism activity of novel glitazones derivatives on haloperidol induced Parkinson activity in mice (SOD, GSH, LPO)

Groups	SOD	GSH	LPO
Normal	4.18±0.06	1.81±0.07	2.20±0.04
Control	0.60±0.04[a]	1.03±0.05[a]	3.82±0.08[a]
Standard	1.60±0.07[a, b]	1.14±0.04[a]	2.30±0.08[b]
C_{10} D1+HP	1.25±0.04[a, b, c]	1.15±0.10[b]	2.37±0.07[b]
C_{10} D2+HP	2.13±0.04[a, b, c]	1.53±0.11[b]	2.29±0.06[b]
C_{12} D1+HP	1.13±0.04[a, b, c]	0.90±0.10[a]	2.45±0.07[b]
C_{12} D2+HP	2.01±0.05[a, b, c]	1.25±0.05[a]	2.53±0.07[b]

All values are expressed as Mean ± SEM n=6. The antioxidant levels were analysed by One-way ANOVA followed by Tukey's multiple comparison test [a]P<0.05 compared to normal [b]P<0.05 compared to control [c]p<0.05 compared to standard.

Estimation of SOD

The endogenous antioxidant levels are mainly responsible for the anti-Parkinson's activity in the brain. SOD was significantly decreased in the control group (0.6±0.04) when compared when compared with the normal group (4.18±0.06) hence it showed the significant induction of the PD. The SOD levels were increased in the test group when compared with the control group. Standard treatment (1.60±0.07). The compound C10(1.25±0.04) and C12 (1.13±0.04) at the dose 10mg/kg has showed significant decrease in SOD levels when compared with standard group (1.60±0.07) where has, at the dose of 20mg/kg showed significant increase in the SOD levels when compared with the standard group. Test compound C10 at dose 20mg/kg showed increased levels of SOD when compared with C12.

Estimation of GSH

The endogenous antioxidant levels are mainly responsible for the anti-Parkinson's activity in the brain. GSH was significantly decreased in the control group (1.81±0.07) when compared when compared with the normal group (1.03±0.05) hence it showed the significant induction of the PD. The GSH levels were increased in the test group when compared with the control group. Standard treatment (1.14±0.04). The compound C10 (1.15±0.10) showed same levels as that of standard and C12 (0.90±0.10) at the dose 10mg/kg has showed significant decrease in GSH levels when compared with standard group (1.14±0.04). At the dose of 20mg/kg showed significant increase in the GSH levels when compared with the standard group. Test compound C10 at dose 20mg/kg showed increased levels of GSH when compared with C12.

Estimation of LPO

The endogenous antioxidant levels are mainly responsible for the anti-Parkinson's activity in the brain. LPO was significantly increased in the control group (3.82±0.08) when compared when compared with the normal group (2.20±0.04) hence it showed the significant induction of the PD. The LPO levels were decreased in the test group when compared with the control group. When compared the standard group (0.90±0.10) with test compound C10 (2.37±0.07), (2.29±0.06) and C12 (2.45±0.07), (2.53±0.07) showed very less significant difference between two groups. The test compound C10 at dose of 20mg/kg showed less difference in the decreased levels of LPO when compared with compared with C12.

DISCUSSION

In the present study anti-Parkinson's activity of novel glitazones were screened in haloperidol induced PD model. In the present study, animals were treated with haloperidol (1mg/kg) through intraperitonially for 7days showed the cataleptic behaviour similar to the symptoms of PD. In this study we evaluated anti-parkinsonism activity of two novel glitazones C10 and C12.

The different binding interactions of PPAR with the different ligands were found to be Met 106(A), Asp 104(A), and Lys 92(A) etc. Among which the potential binding sites were Lys 112(A), Asp 109(A), Met 106, from which most of the ligands showed hydrogen bond interactions. The hydrogen distances were in the range of 1.3 to 2.9 A^{o}. The different binding sites were obtained because of the diversity in the structures of the binding ligands with respect to the reference ligand.

In the rotarod, an animal walk on a rotating rod, and is widely used to assess motor coordination skills of animals. The performance is measured by the duration in seconds that an animal stays up on the rotating rod as a function of drum speed[4].

In the present study, normal group showed 51.3% increased latency fall of time when compared with control group. Whereas at dose 10mg/kg and 20mg/kg compound C10 showed 33.8% and 48.1% increase in the fall of time when compared with control group. The compound C12 at dose 10 and 20mg/kg showed 33.1% and 46% increase in the fall of time when compared with control group. The standard group showed 6.2% and 7.3% increased latency fall of time when compared with C10 and C12 at dose 10mg/kg. Whereas at dose 20mg/kg C10 and C12 showed 16.4% and 13.1% increased latency fall of time when compared with standard group. The C10 at dose 10mg/kg showed 1.2% increased latency fall of time when compared with C12. The C10 at dose 20mg/kg showed 3.9% increased latency fall of time when compared with C12.

In the photo actometer (SLA), photocell is activated when the rays of light falls on the photocells are cut-off by animals crossing the beam of light. The performance is measured by the intensity of locomotory activity[5].

In the present study, normal group showed 75.9% increased locomotory activity when compared with control group. Whereas at dose 10mg/kg and 20mg/kg compound C10 showed 69.3% and 73.7% increased locomotory activity when compared with control group. The compound C12 at dose 10 and 20mg/kg showed 37.9% and 67.5% increased locomotory activity when compared with control group. The C10 at dose 10mg/kg showed 45.4% increased locomotory activity when compared with standard group, where the standard group showed 9.5% increased locomotory activity when compared with C12 at 10mg/kg. The C10 and C12 showed 53.3% and 42.3% increased locomotory activity at dose 20mg/kg showed 1.2% increase when compared with standard group. The C10 at dose 10mg/kg showed 50.6% increased locomotory activity when compared with C12. The C10 at dose 20mg/kg showed 19.1% increased locomotory activity when compared with C12.

The staircase mainly consists of 5 steps where an animal was placed near the steps and the time taken to climb the steps was counted to evaluate the anti-parkinsonism activity. In this study we had given 1 week of training before inducing the drug where the evaluation was done on the last day of the study[6].

In the present study, control group has taken 79.65% of more time to show muscle activity when compared with normal group. Whereas at dose 10mg/kg and 20mg/kg compound C10 has taken 55.4% and 60.4% of less time to show muscle activity when compared with control group. The compound C12 at dose 10 and 20mg/kg
Has taken 43.1% and 45% of less time to show muscle activity when compared with control group. The standard group has taken 18.8% and 36.3% of less time to show muscle activity when compared with C10 and C12 at dose 10mg/kg. The standard group taken 8.7% and 34.1% muscle activity when compared with C10 and C12 at dose 10mg/kg. The C10 at dose 10mg/kg has taken 21.6% less time to show muscle activity when compared with C12. The C10 at dose 20mg/kg has taken 27.9% less time to show muscle activity when compared with C12.
In order to access the antioxidant effect of both the compounds C10 and C12 on haloperidol induced PD. The antioxidant levels were estimated in the brain.

In the present study, normal group showed 85.4% increased SOD levels when compared with control group. Whereas at dose 10mg/kg and 20mg/kg compound C10 showed 50% and 45.5% increased SOD levels when compared with control group. The compound C12 at dose 10 and 20mg/kg showed 71.5% and 70% increased SOD levels when compared with control group. The standard group showed 25% and 31.2% increased SOD levels when compared with C10 and C12 at dose 10mg/kg. The C10 and C12 showed 23.9% and 20% increased SOD levels when compared with standard group. The C10 at dose 10mg/kg showed 9.6% increased SOD levels when compared with C12. The C10 at dose 20mg/kg showed 5.7% increased SOD levels when compared with C12.

In the present study, normal group showed 43.1% increased GSH levels when compared with control group. Whereas at dose 10mg/kg and 20mg/kg compound C10 showed 10.5% and 32.7% increased GSH levels when compared with control group. The compound C12 at dose 10mg/kg showed 12.7% decreased GSH levels and at 20mg/kg showed 17.6% and 70% increased GSH levels when compared with control group. The standard group showed 0.9% and 25.5% decreased GSH levels when compared with C10 and C12 at dose 10mg/kg. The standard group showed 12.7% increased GSH levels when compared with C12 at dose 10mg/kg, whereas standard group showed 8.8% decreased GSH levels when compared with C12 at dose 20mg/kg. The C10 at dose 10mg/kg showed 21.8% increased GSH levels when compared with C12. The C10 at dose 20mg/kg showed 18.4% increased GSH levels when compared with C12.

The haloperidol causes the over turnover of the dopamine this leads to the extrapyramidal effects and causes increased oxidative stress.
In the present study, normal group showed 42.2% decreased LPO levels when compared with control group. Whereas at dose 10mg/kg and 20mg/kg compound C10 showed 38% and 40.1% decreased levels when compared with control group. The compound C12 at dose 10 and 20mg/kg showed 35.9% and 33.8% decreased LPO levels when compared with control group. The standard group showed 3% decreased LPO levels when compared with C10 at dose 10mg/kg. The C10 at 20mg/kg showed 0.5% decreased LPO levels when compared with standard group. The C12 at dose 10 and 20mg/kg showed 6.2% and 9.1% increased LPO levels when compared with standard group. The C10 at dose 10mg/kg showed 3.3% decreased LPO levels when compared with C12. The C10 at dose 20mg/kg showed 9.5% decreased levels of LPO when compared with C12.

SUMMARY AND CONCLUSION

We have studied the effect of novel glitazones on Parkinson's disease animal model and showed that it can be used for the treatment of PD. The evaluation was done by using rotarod, actophotometer and stair case, the parameters used to evaluate were spontaneous locomotory activity, muscle strength and gripping action respectively. The novel glitazone had shown antioxidant and reduced oxidative stress. Both the compounds exhibited dose dependent activity, however C10 at higher dose showed best activity when compared with C12. But further studies are required to support the present assumption and elucidate detailed neuroprotective mechanism.

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