World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 6, Suppl 2, 2017 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article Full Proceeding Paper

MOLECULAR CHAPERONES AS THERAPEUTIC TARGETS FOR PARKINSONISM AND INFLAMMATION

Dr. M. Ganga Raju *, Anusha K, P. Venkanna, Sayan Dutta Gupta

Department of pharmacology, Gokaraju Rangaraju College of Pharmacy, Hyderabad -500090, Telangana, INDIA.

Received on: 05-10-2017; Revised and Accepted on: 08-11-2017

ABSTRACT

Heat-shock protein (Hsp90) is one of a group of molecular chaperones responsible for managing protein folding and quality control in cell environment. Hsp90 requires a series of co-chaperones to assemble into a super-chaperone complex for its function. The present study is an attempt to explore the role of Hsp90 in various activities like anti-Parkinson's and anti-inflammatory activities. In the present study the two test compounds C1 and C2 were synthesized and the acute toxicity dose was found to be 550 mg/kg bd.wt. The anti-Parkinson's activity was performed using reserpine induced and haloperidol induced Parkinson's model using bromocriptine as standard drug. In this study the animals were administered with a dose of 50 mg/kg bd.wt and 100 mg/kg bd.wt and compared with control and standard groups. The anti-inflammatory activity was performed using carrageenan induced and formalin induced paw edema model taking indomethacin as standard drug. The results were compared with control and standard groups. The two test compounds C1 and C2 has shown significant amelioration of Parkinson's disease symptoms like bradykinesia, palpebral ptosis, tremor, righting reflex,muscular rigidity, catalepsy, locomotor activity in both reserpine induced and haloperidol induced Parkinson disease. These schiff's bases C1 & C2 are potential inhibitors of Hsp 90 and these might have reduced the above symptoms by inhibiting Hsp 90 with subsequent activation of HSF-1 (heat shock factor-1) and production of Hsp 70 and Hsp 40. These two chaperones reduce the expression of α-synuclein with consequent reduction in fibrils formation and neurodegeneration. In anti-inflammatory activity the test compound have shown inhibition in paw volume. Administration of two test compounds C1 and C2 to carrageenan and formalin induced rats a dose dependent inhibition of paw volume was observed and this might be due to the blocking e-NOS through Hsp 90 inhibition. From the results it was found that the two test compounds C1 and C2 possess anti-Parkinson's and anti-inflammatory activities.

KEYWORDS: *Hsp 90 inhibitors, anti-Parkinson's, anti-inflammatory, Bromocriptine.*

INTRODUCTION

Parkinson's (PD) is the second most common neurodegenerative disorder affecting approximately 1% of the population over 60 [1]. People with PD typically present with cardinal motor symptoms including bradykinesia, muscular rigidity, rest tremor, or gait impairment but often also develop nonmotor symptoms, such as cognitive impairment and psychiatric symptoms. Currently, PD is treated pharmacologically, by enhancing dopamine tone (e.g. dopamine replacement with L-dopa) and surgically by deep brain stimulation (DBS) [2]. Pathologically, PD is characterized by the presence of proteinaceous intracellular aggregates composed primarily of *α*-synuclein, termed Lewy pathology (Lewy bodies and Lewy neurites). Missense mutations and multiplications of the *SNCA* gene, which encodes for *α*-synuclein, cause heritable forms of PD and enhance the propensity of *α*-synuclein to self-aggregate thus implicating *α*-synuclein aggregation in the pathogenesis of the disease [3, 4].

Inflammation is an important component of treatment-refractory inflammation is a feature of many chronic degenerative diseases. Hsp90 is a 90kDa protein which functions as an ATP-dependent molecular chaperone that regulates the signalling conformation and expression of multiple protein client proteins especially oncogenic mediators. Hsp90 inhibitors are in clinical development as cancer therapies but the myelosuppresssive and neutropenic effect of first generation geldanamycin-class inhibitors has confounded studies on the effects on Hsp90 inhibitors on inflammation. Ganetespib also suppressed B cell and NK cell accumulation, inflammatory cytokine and chemokine induction and MMP9 levels. These data identify non-myelosuppresssive Hsp90 inhibitors as potential therapies for inflammatory diseases refractory to conventional therapy [5]. Hsp90 associates with endothelial nitric oxide synthase (eNOS) and is rapidly recruited to the eNOS complex by agonists that stimulate production of nitric oxide, namely vascular endothelial growth factor, histamine and fluid shear stress.

Endothelial nitric oxide (eNOS) has recently been recognized as client of Hsp90. Hsp90 is physically associated with eNOS in resting endothelial cells. Activation of endothelial cells by vascular endothelial growth factor, histamine, fluid shear stress and estrogen enhances the interaction between eNOS and Hsp90 thus increasing eNOS activity. However, eNOS, which produces nanomolar levels of NO, also plays a role in inflammation. Endothelial nitric oxide for example, can regulate the expression of the pro-inflammatory molecules nuclear factor-kB (NF-kB) and cyclooxygenase-2.

Currently, no curative treatments or treatments that interdict disease progression exist. Although the etiology of PD remains unknown, abundant evidence implicates immune system abnormalities and central nervous system (CNS) inflammation in disease pathobiology [6, 7]. Harnessing inflammatory responses through targeted modulation of innate and adaptive immune responses has gained increasing interest in recent years as a potential therapeutic strategy. The interplay between innate and adaptive immunity in the pathobiology of PD, the evolution and change in such immune responses and the means to alter it to the benefit of the diseased is the focus of this article.

***Corresponding author:**

Dr. M. Ganga Raju

Department of pharmacology,

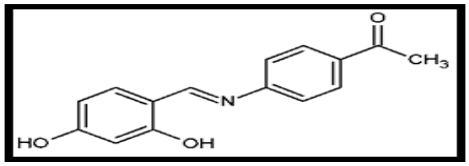
Gokaraju Rangaraju College of Pharmacy,

Hyderabad -500090, Telangana, INDIA.

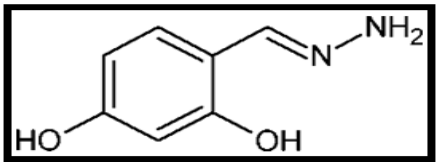
MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goals under the prescribed guidelines and recommendations. It includes various steps in the synthesis of drug, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, formation of protocols and final execution of the standardized protocol.

Structure of C1 and C2:



4-(Hydrazonomethyl)benzene -1,3 diol



4-(-amino benzophenone) methyl) benzene 1,3 diol

Fig. 1: Structure of C1 and C2

Animal procurement:

Female Sprague Dawley rats (below 50 days age) and Swiss albino mice were obtained from Albino Research & Training Institute, Hyderabad. They were maintained on a standard pellet diet and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions (12 h light–dark cycle; 23–25°C; 35–60% relative humidity). All the experimental protocols for animal care procedures were approved by the Institute Animal Ethics Committee (IAEC). Principles of laboratory animal care guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee (IAEC) for conducting the experiments. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India (Reg. no.1175/PO/Ere/S/08/CPCSEA).

Acute toxicity testing:

Studies were carried out in order to check the toxic effects of the test compounds. The study was performed as per Organization for Economic Cooperation and Development (OECD). The method used to evaluate the acute oral toxicity is by up and down procedure (OECD guideline-425).

Anti-Parkinson's Activity:

Antiparkinson's activity was screened using two models like reserpine induced and haloperidol induced Parkinson's disease.

Reserpine induced Parkinson's disease:

Reserpine is an indole alkaloid, derived from *Rauwolfia serpentina* root and bark. Reserpine induced Parkinson is relatively good model of the disease biochemistry. Most attention has nevertheless been paid to the dopaminergic deficit, and it is known that reserpine produces 85% loss of dopamine in the SNpc and >95% dopamine depletion in the striatum within 2 h of injection. Although dopamine content in the SNpc returns to 30% by 24 h post injection, striatal dopamine depletion persists at >95% for at least 24 h. Behaviourally, reserpine induces features of akinesia and hind limb rigidity in rats that are representative of symptoms associated with Parkinson's disease [8].

Procedure:

Swiss mice of either sex weight 20-25g were selected for the present study obtained from Albino research & training institute, Hyderabad. They were housed in polypropylene cages with bedding and maintained at 25 °C and 50 % humidity with 12 h light-dark cycle. The animals received an *intra peritoneal* injection (*i.p.*) of 5 mg/kg bd. wt of reserpine. Mice were treated with reserpine for 5 consecutive days to generate acute dopamine depletion. The test compounds and standard drugs administered 30 min before recording the observations. Bromocriptine (2.5 mg/kg) was used as standard drug. A total of 42 mice were divided into seven groups with six animals in each group [8].

Haloperidol induced Parkinson's model:

Haloperidol works by antagonizing dopamine D2 and to lesser extent D1 receptors in medium spiny neurons that comprise the indirect and direct pathways of the motor circuit respectively. The resultant block of striatal dopamine transmission results in abnormal downstream firing within the basal ganglia circuits that manifests as symptoms of muscle rigidity and catalepsy within 60 min of haloperidol (0.5-5 mg/kg, *i.p.*) injection. Although rigidity is a feature of Parkinson's disease, providing this model with some face validity, catalepsy, which is expressed as the inability of an animal to correct itself from an abnormally imposed posture, is not directly associated with Parkinson's disease.

Procedure:

This model may provide novel approaches for the development of drugs, which can reverse cataleptic state in patients with PD and can reverse or prevent the extrapyramidal side effects associated with antipsychotic treatment [9]. After 30 min of administration of haloperidol (1 mg/kg, *i.p.*), the duration of catalepsy was measured for 5 min at an interval of 30, 60, 90, and 120 min. Duration of catalepsy was determined by placing an animal on the horizontal metal bar at a height of 6 cm in such a way that the fore-limbs of the animal should be on the horizontal bar while the hind-limb touches the surface. The animals were treated with MEBV (100, 200, and 300 mg/kg, *p.o.* respectively), or L-Dopa-carbidopa (30 mg/kg, p.o.) 60 min prior administration of haloperidol.

Anti-inflammatory activity:

Anti-inflammatory activity was carried out using two models like carrageenan induced paw edema and formalin induced paw edema model.

Carrageenan-induced paw edema model:

The anti-inflammatory activity was assessed using carrageenan-induced paw edema in the rat model. Here female Sprague Dawley rats are used. Acute paw edema was induced by *sub-plantar* injection of carrageenan suspension (0.1 ml of 1% w/v freshly prepared suspension in normal saline) into the right hind paw of each rat. The left hind paw was injected with the same volume of 0.1 ml of normal saline. Indomethacin (10 mg/kg bd.wt dose orally) was used as standard drug. Rats were pre-treated with test compounds C1 & C2 and standard drug 1 h prior to carrageenan administration [10]. The paw size was measured in mm using Plethysmometer before (Initial paw volume) and at 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after carrageenan administration. A total of 42 rats were used. The rats were divided into seven groups with six animals in each group.

Formalin-induced paw edema model:

The anti-inflammatory activity was assessed using formalin-induced paw edema in the rat model. Here female Sprague Dawley rats are used. Acute paw edema was induced by *sub-plantar* injection of formalin solution (1% w/v freshly prepared solution in normal saline) into the right hind paw of each rat. The left hind paw was injected with the same volume of 0.1 ml of normal saline. Indomethacin (10 mg/kg bd.wt.*p.o*) was used as standard drug. Rats were pre-treated with test compounds C1 and C2 and standard drug 1 h prior to formalin administration. The paw size was measured in mm using plethysmometer before (0 min) and at 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after formalin administration. A total of 42 rats were used. The rats were divided into seven groups with six animals in each group [11].

RESULTS AND DISCUSSION

The two test compounds C1 and C2 belonging to Hsp90 inhibitor class have been synthesized. The structures of synthesized compounds C1 and C2 are well supported by spectral data such as NMR and Mass spectral analysis. The synthesized compounds were screened for anti-Parkinson's and anti-inflammatory activity and the results were reported.

Acute toxicity studies:

The test compounds showed no signs of toxicity or mortality upto 550 mg/kg but exerted toxicity at 2000 mg/ kg. All animals were safe and there was no reduction in body weight upto 14 days when treated with different doses upto 550 mg/kg, bd.wt. Hence the test compounds were found to be safe upto 550 mg/kg, bd.wt.

Anti-Parkinson's activity:

Reserpine induced Parkinson's model:

The anti-Parkinson's activity was performed using reserpine induced Parkinson's model. The various parameters like bradykinesia, Palpebral ptosis, tremor, righting reflex, muscular rigidity, catalepsy, locomotor behaviour were measured. The values were reported in the Table 1 and Table 2.

Reserpine treatment showed increase in time taken to remove both forepaws from the metal rod when compared to control group and bromocriptine. Treatment with test compounds C1 and C2 at doses of 50 mg/kg and 100 mg/kg, standard drug bromocriptine (2.5 mg/kg) significantly reduced this removal time. Significant increase in palpebral ptosis score was observed in reserpine induced group when compared to control group. Treatment with test compounds C1, C2 and bromocriptine at a dose of 2.5 mg/kg significantly reduced this score compared to reserpine induced group. The administration of reserpine significantly increased tremors when compared to control. Treatment with test compounds C1 &C2 significantly reduced the tremors compared to reserpine induced group. Both the test compounds C1 and C2 showed the effect and the activity of C2 were found to be better than that of C1.Reserpine administration resulted in significant increase righting reflex score when compared to control group. Treatment with test compounds C1 and C2 at doses of 50 mg/kg and 100 mg/kg and bromocriptine significantly reduced the score compared to reserpine induced group.

Reserpine reduced the muscle rigidity when compared to control group. The test compound C1and C2at two doses 50 mg/kg and 100 mg/kg has shown muscular rigidity when compared to control group. From the above results the two test compound C1 & C2 at a dose of 100 mg/kg has shown significant activity when compared to that of standard drug bromocriptine. Significant increase in cataleptic score was observed in reserpine induced group when compared to control group. The reserpine induced catalepsy was significantly reduced by both the test compounds and bromocriptine. Significant reduction in locomotor activity was observed in reserpine induced group when compared to control group. Treatment with test compounds (C1 and C2) and bromocriptine significantly improved the locomotor activity compared to reserpine induced group. Reserpine treatment significantly attenuated the basal activity score when compared to normal control group. Treatment with test compounds and bromocriptine significantly improved the basal activity score compared to reserpine induced group. Reserpine treatment significantly decreased swimming activity of mice in reserpine induced group compared to control group. Administration of test compounds and standard significantly improved swimming time compared to reserpine induced group.

Table No. 1: Reserpine induced Parkinson's activity of test compounds C1 and C2 in Swiss albino mice

Groups	Bradykinesia (sec)	Palpebral Ptosis (score)	Tremor (score)	Righting reflex (score)
Control	1.50±1.78	0.00±0.00	0.00±0.00	0.00±0.00
Reserpine induced	12.33±0.22 a	3.50±0.22 a	2.83±0.22 a	7.50±0.22 a
C1-50 mg/kg	10.50±0.33 *C	3.33±0.42 **,B	2.66±0.22 **,A	6.16±0.22 **,B
C1-100 mg/kg	8.33±0.50 **A	2.83±0.21 ***,A	2.33±0.21 **,B	5.50±0.22 ***A
C2-50 mg/kg	9.33±0.22 **B	3.33±0.40 **,B	2.50±0.22 **,B	5.16±0.22 ***B
C2-100 mg/kg	6.83±0.60a ***A	2.66±0.21 ***,A	2.16±0.21 ***,A	4.33±0.22 ***A
Bromocriptine 2.5 mg/kg	4.33±0.66 a***	2.50±0.67 a***	2.0±0.44 a***	2.83±0.21 a***

*Values were expressed as mean ±SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's t-test by comparing with normal control, reserpine induced and standard. Significant values were expressed as control group (a=p<0.001, b=p<0.01, c=p<0.05), negative control (***=p<0.001, **=p<0.01, *=p<0.05) and standard (A=p<0.001, B=p<0.01, C=p<0.05).*

Table No. 2: Reserpine induced Parkinson's activity of test compounds C1 and C2 in Swiss albino mice

Groups	Muscular rigidity (sec)	Catalepsy (score)	Locomotor behavior (sec)	Basal activity (sec)	Swim test (sec)
Control	57.0±1.23	0.00±0.00	130.33±11.13	62.83±3.74	58.33±0.25
Reserpine Induced	6.0±0.36 a	2.66±1.38 b	25.0±1.82 a	8.16±0.30 a	12.16±1.01 a
C1-50 mg/kg	26.33±1.03 **B	2.25±0.25 **,B	45.33±1.28 ***B	13.66±0.44 ***B	39.66±2.24 **B
C1-100 mg/kg	30.33±2.62 ***A	1.66±0.15***B	52.33±2.02***A	18.66±1.20 ***A	49.66±2.16 ***A
C2-50 mg/kg	27.00±1.64 ***B	1.75±3.80 ***B	46.5±0.99 ***B	17.60±3.91 **A	47.66±1.8 ***A
C2-100 mg/kg	42.16±2.76 ***,A	1.25±1.85***A	56.33±0.55 ***A	20.66±0.21 ***A	52.87±2.41 ***A
Bromocriptine 2.5 mg/kg	46.16±2.62 a***	0.5±.2.45***a	85.0±3.16 ***a	24.66±0.76 a***	56.33±1.8 ***,a

*Values were expressed as mean ±SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnet's t-test by comparing with normal control, reserpine induced and standard. Significant values were expressed as control group (a=p<0.001, b=p<0.01, c=p<0.05), negative control (***=p<0.001, **=p<0.01, *=p<0.05) and standard (A=p<0.001, B=p<0.01, C=p<0.05).*

The reserpine induced Parkinson's model is suitable for evaluating anti-Parkinson's drugs. Reserpine irreversibly blocks the vesicular monoamine transporter. This depletion of dopamine can lead to drug-induced Parkinsonism. In this model C1 and C2 shown significant reduction in symptoms when compared with the standard drug bromocriptine [12].

The most important neuropathological hallmark of Parkinson's disease is considered to be the death of the dopaminergic neurons in the substantial nigra pars compacta (SNpc). Nigral neurodegeneration result in a reduction of the neurotransmitter dopamine in the striatum, the main target of the SNpc, causing dysregulation within the basal ganglia. The presence of intracellular protein aggregates, known as Lewy bodies and Lewy neurites, within

the surviving dopaminergic neurons of the SNpc is the defining neuropathological feature of the disease. Lewy bodies and Lewy neurites contain a number of proteins and often highly ubiquitinylated. 3-Synuclein is a major component of the filaments of Lewy bodies and Lewy neurites.

The expression of this 3-Synuclein is majorly regulated by molecular chaperones and co-chaperones including Hsp90, Hsp70, Hsp40 etc. Hence prevention of 3-Synclein expression by modulating heat shock proteins is an effective mode of treatment of neurodegenerative Parkinson's disease.

The test compounds C1 and C2 showed significant amelioration of Parkinson's disease symptoms like bradykinesia, palpebral ptosis, tremor, righting reflex, muscular rigidity, catalepsy, locomotor activity and swim test. These Schiff's bases C1 and C2 are potential inhibitors of Hsp90. Hence they might have reduced the above symptoms by inhibiting Hsp90 with subsequent activation of HSF-1 (heat shock factor-1) and production of Hsp70 and Hsp40. These two chaperones reduce the expression of α-synuclein with consequent reduction in fibrils formation and neurodegeneration [13].

Haloperidol induced Parkinson's disease:

The anti-Parkinson's activity was performed using haloperidol induced Parkinson's model. The various parameters like bradykinesia, Palpebral ptosis, tremor, righting reflex muscular rigidity, catalepsy, locomotor behaviour and swim test were measured. The values were reported in the Table 3 and 4.

Haloperidol treatment showed increase in time taken to remove both forepaws from the metal rod when compared to control group and bromocriptine treated group. Treatment with test compounds C1 and C2 significantly reduced this removal time, both the test compounds exhibited dose dependent effect and the activity of C2 at dose of 100 mg/kg bd.wt.was found to be better than that of C1 at dose of 50 mg/kg bd. wt. Significant increase in palpebral ptosis score was observed in haloperidol induced group compared to control group. Treatment with test compounds and bromocriptine significantly reduced this score compared to haloperidol induced group. The administration of haloperidol significantly increased tremors compared to control group. Treatment with test compounds significantly reduced the tremors compared to haloperidol induced group. Both the test compounds showed the effect and the activity of C2 was found to be better than that of C1. Haloperidol administration resulted in significant increase righting reflex score when compared to control group. Treatment with test compounds C1, C2 and Bromocriptine significantly reduced the score compared to haloperidol induced group.

Haloperidol treatment significantly decreased the time taken to fall from metal rod when compared to control group indicating induction of high muscular rigidity. Administration of test compounds C1 and C2 showed significant increase in fall of time which is an index of reduced muscular rigidity. Haloperidol administration produced significant increase in cataleptic score compared to control group. The haloperidol induced catalepsy was significantly reduced by both the test compounds and bromocriptine. C1 and C2 produced dose dependent effect and the activity of C2 was found to be better than that of C1. The effect of test compound C2 at dose of 100 mg/kg bd,wt. was comparable to that of standard drug bromocriptine. Significant reduction in locomotor activity was observed in haloperidol induced group when compared to control group after administration of haloperidol. Treatment with test compounds C1, C2and bromocriptine significantly improved the locomotor activity compared to haloperidol induced group. The effect of compounds under investigation was found to be dose dependent and C2 produced better effect than C1.Haloperidol treatment significantly attenuated the basal activity score in haloperidol induced group when compared to normal control group. Treatment with test compounds and bromocriptine significantly improved the basal activity score compared to haloperidol induced group. Both test compounds showed dose dependent effect and the effect of C2 was found to be better than that of C1. The activity of test compound C2 at dose of 100 mg/kg bd, wt. was comparable to that of standard drug bromocriptine. Haloperidol treatment significantly decreased swimming activity of rats in haloperidol induced group when compared to control group. Administration of test compounds and standard significantly improved swimming time compared to haloperidol induced group.

Table No. 3: Haloperidol induced Parkinson's activity of test compounds C1 and C2 in Sprague Dawley rats

Groups	Bradykinesia (sec)	Palpebral ptosis (score)	Tremor (score)	Righting reflex (score)
Control	1.50±1.19	0.00±0.00	0.00±0.00	0.00±0.00
Haloperidol induced	10.33±0.2 a	3.50±0.22 a	3.0±0.00 a	7.50±0.00 a
C1-50 mg/kg	9.16±0.22 **B	2.33±0.21 **	2.50±0.21 **,B	6.00±0.21 **,C
C1-100 mg/kg	7.33±0.61 **A	1.83±0.31 ***,A	1.66±0.22 ***,A	4.83±0.22 ***A
C2-50 mg/kg	8.50±0.22 ***B	2.16±0.21 **,B	2.33±0.22 **,B	5.66±0.16***B
C2-100 mg/kg	5.33±0.22 ***A	1.33±0.21 ***,A	1.50±0.21 ***,A	3.66±0.21 ***A
Bromocriptine 2.5 mg/kg	3.33±0.99***,a	0.83±0.36 a***	1.33±0.21 a***	1.50±0.22 a***

*Values were expressed as mean ±SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's t-test by comparing with normal control, reserpine induced and standard. Significant values were expressed as control group (a=p<0.001, b=p<0.01, c=p<0.05), negative control (***=p<0.001, **=p<0.01, *=p<0.05) and standard (A=p<0.001, B=p<0.01, C=p<0.05).*

Table No.4: Haloperidol induced Parkinson's activity of test compounds C1 and C2 in Sprague Dawley rats

Groups	Muscular rigidity (sec)	Catalepsy (score)	Locomotor behavior (sec)	Basal activity (sec)	Swim test (sec)
Control	59.33±1.05	0.00±0.00	124.66±0.91	67.66±0.91	56.16±0.16
Haloperidol Induced	3.50±0.22 a	2.25±1.28 b	48.33±1.01 a	27.16±1.01 a	23.33±0.25 a
C1-50 mg/kg	13.66±0.33 ***C	2.16±1.22 *c	62.16±1.35 **B	33.00±1.35 **,C	38.16±0.33 **B
C1-100 mg/kg	20.50±0.50 ***A	1.66±1.54**A	88.50±.0.90 ***A	40.16±.0.90 ***A	48.33±0.50 ***A
C2-50 mg/kg	18.33±0.61 **B	1.50±0.98**B	75.16±0.77 ***B	34.16±0.77 **,B	40.16±0.90 ***B
C2-100 mg/kg	25.50±0.50 **A	1.25±1.11**,B	99.83±1.44 ***A	42.16±1.44 ***,A	51.16±0.12 ***A
Bromocriptine 2.5 mg/kg	43.10±0.74a***	1.05±3.60a***	106.16±0.91 a***	52.66±0.91 a***	54.33±0.25 ***a

*Values were expressed as mean ±SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's t-test by comparing with normal control, reserpine induced and standard. Significant values were expressed as control group (a=p<0.001, b=p<0.01, c=p<0.05), negative control (***=p<0.001, **=p<0.01, *=p<0.05) and standard (A=p<0.001, B=p<0.01, C=p<0.05).*

The haloperidol induced Parkinson's model are suitable for evaluating anti-Parkinson's drugs. Haloperidol is a butyrophenone derivative and functions as an inverse agonist of dopamine. Haloperidol, a neuroleptic drug blocks the action of dopamine which may result in Parkinson's disease is one of the major causes for drug induced Parkinson's. It blocks dopamine D2 receptor and produces a state of catalepsy in human or animals by reducing dopaminergic transmission in basal ganglion. In this model C1 and C2 shown significant reduction in symptoms when compared with the standard drug bromocriptine.

The most important neuropathological hallmark of Parkinson's disease is considered to be the death of the dopaminergic neurons in the substantial nigra pars compacta (SNpc). Nigral neurodegeneration result in a reduction of the neurotransmitter dopamine in the striatum, the main target of the SNpc, causing dysregulation within the basal ganglia. The presence of intracellular protein aggregates, known as Lewy bodies and Lewy neurites, within the surviving dopaminergic neurons of the SNpc is the defining neuropathological feature of the disease. Lewy bodies and Lewy neurites contain a number of proteins and often highly ubiquitinylated. 3-Synuclein is a major component of the filaments of Lewy bodies and Lewy neurites. The expression of this 3-Synuclein is majorly regulated by molecular chaperones and co-chaperones including Hsp90, Hsp70, Hsp40 etc. Hence prevention of 3-Synclein expression by modulating heat shock proteins is an effective mode of treatment of neurodegenerative Parkinson's disease.

The test compounds C1 and C2 showed significant amelioration of Parkinson's disease symptoms like bradykinesia, palpebral ptosis, tremor, righting reflex, muscular rigidity, catalepsy, locomotor activity and swim test. These Schiff's bases C1 and C2 are potential inhibitors of Hsp90. Hence they might have reduced the above symptoms by inhibiting Hsp90 with subsequent activation of HSF-1 (heat shock factor-1) and production of Hsp70 and Hsp40. These two chaperones reduce the expression of α-synuclein with consequent reduction in fibrils formation and neurodegeneration [13].

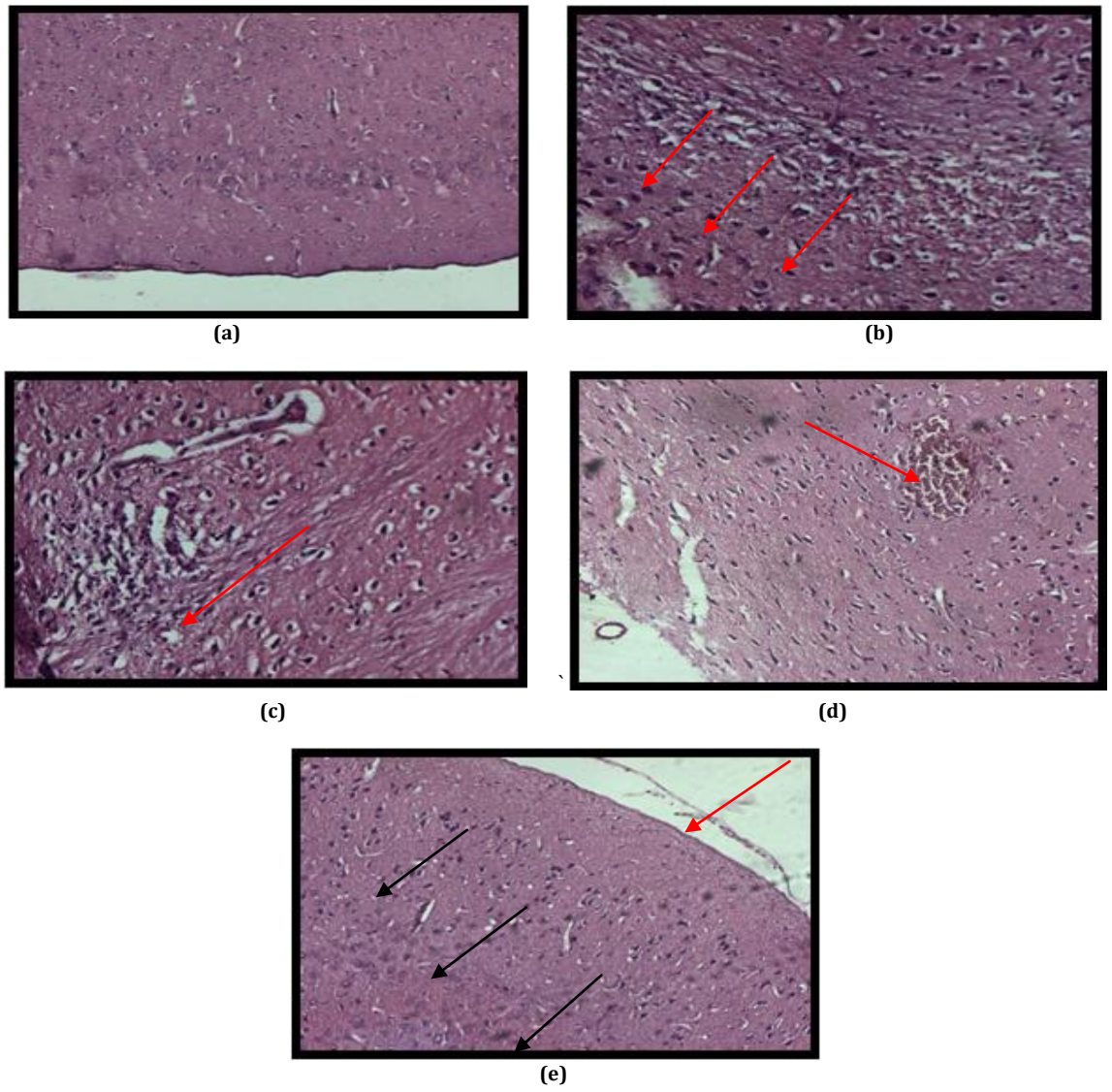


Fig. 2: Effect of C1 and C2 on histopathological changes in the brain of normal and haloperidol treated animals (H&E staining; original magnification, 40x). *(a) Normal control showing normal brain architecture. (b) Rats treated haloperidolshowed Mild to moderate demyelination and necrosis noticed in the cerebral hemisphere. (c) Rats treated with haloperidol and C1 (100 mg/kg) showed mild foci of necrosis and inflammation noticed in the cerebral hemisphere. (d) Rats treated with haloperidol and C2 (100 mg/kg) showed foci of haemorrhage or hematoma formation noticed in the cerebral hemisphere of brainand (e) Rats treated with haloperidol and bromocriptine (2.5 mg/kg) showed cerebral hemisphere and meninges surrounding the cerebral hemisphere appeared normal.*

Hence the anti-Parkinson's activity of the compounds might be due to the inhibition of Hsp90 thereby preventing the protein disaggregation and degradation. By this demyelination of the neurons in the cerebral hemisphere was prevented and dopaminergic activity was restored.

Anti-inflammatory activity:

Carrageenan induced inflammatory activity:

The anti-inflammatory activity was performed using carrageenan induced paw edema model. The paw volume at 1 h, 2 h, 3 h, 4 h, 5 h and 6 h were measured and the paw volume percentage inhibition in paw volume, the intial paw volume and final paw volume were calculated. The percentage inhibition of paw volume were reported in the Table 5. The percentage inhibition in paw volume of the test compounds C1 at a dose of 50 mg/kg bd.wt and 100 mg/kg bd.wt were found to be 66.66% and 71.4% respectively. The percentage inhibition in paw volume of the test compound C2 at a dose of 50 mg/kg bd.wt and 100 mg/kg bd.wt were 60% and 64.28%. The standard drug indomethacin 10 mg/kg bd.wt has shown 87.50% inhibition in paw volume. The test compounds C1 and C2 have shown significant inhibition in paw volume when compared to control and standard group.

Table No. 5: Percentage inhibition of carrageenan induced anti-inflammatory activity of test compounds C1 and C2 in Sprague Dawley rats

Groups	Treatment	Initial Paw Volume (mL)	Final Paw Volume at 6h (mL)	Difference in Paw Volume (mL)	Percentage Inhibition (%)
I	Control	1.4	1.5	0.1	-
II	Carrageenan induced	1.5	3.4	1.9	-
III	C1-50 mg/kg	1.5	2.0	0.5	66.66
IV	C1-100 mg/kg	1.4	1.8	0.4	71.4
V	C2-50 mg/kg	1.5	2.1	0.6	60
VI	C2-100 mg/kg	1.4	1.9	0.5	64.28
VII	Indomethacin 10 mg/kg	1.4	1.6	0.2	87.50

The Carrageenan-induced rat paw volume is a suitable test for evaluating anti-inflammatory drug. Carrageenan-induced edema is a biphasic response and there are several mediators involved in inflammation. Histamine, serotonin and bradykinin are the first detectable mediators in the first phase of carrageenan-induced inflammation; prostaglandins (PGs) are involved in the increased vascular permeability and are detectable in the second phase of inflammation. Local and/or systemic inflammation is associated with enhanced levels of the pro-inflammatory cytokines, TNF-α, IL-1, and IL-6 [15]. C1 and C2 showed significant dose dependent inhibition in paw volume and elicited anti-inflammatory response comparable with the standard drug indomethacin. Since nitric oxide (NO) has been shown to be involved in the increased vascular permeability in inflammatory experimental model, this activity was performed to investigate whether the test drugs C1 and C2 have anti-inflammatory activity.

Heat shock protein90 (Hsp90) binding to endothelial nitric oxide synthase (eNOS) is an important step in eNOS activation which is the primary source of NO in inflammation. Binding of eNOS to Hsp90 has been shown to occur in response to vascular endothelial growth factor (VEGF), histamine and eNOS activity is increased in a dose-dependent manner in the presence of Hsp90. Interaction between Hsp90 and eNOS enhances the nitric oxide production leads to increased vascular permeability causing inflammation. Administration of two test compounds C1 and C2 to carrageenan induced rats and formalin induced rats have shown a dose dependent inhibition of the paw volume and this might be due to blocking the eNOS through Hsp90 inhibition[14].

Formalin induced inflammatory activity:

The anti-inflammatory activity was performed using formalin induced paw edema model. The paw volume at 1 h, 2 h, 3 h, 4 h, 5 h and 6 h and the percentage inhibition in paw volume, the intial paw volume and final paw volume were calculated. The paw volume and percentage inhibition of paw volume were reported in the Table 6. The percentage inhibition in paw volume of the test compound C1 at a dose of 50 mg/kg bd.wt and 100 mg/kg bd.wt were found to be 66.66% and 50% respectively. The percentage inhibition in paw volume of the test compound C2 at a dose of 50 mg/kg bd.wt and 100 mg/kg bd.wt was 57.14% and 50%. The standard drug indomethacin at a dose of 10 mg/kg has shown 93.75% inhibition in paw volume. The test drugs C1 and C2 have shown significant inhibition in paw volume when compared to control and standard group.

Table No. 6: Percentage inhibition of formalin induced anti-inflammatory activity of test compounds C1 and Dawley C2 in Sprague Dawley rats

Groups	Treatment	Initial Paw Volume(mL)	Final Paw Volume at 6h (mL)	Difference in Paw Volume(mL)	Percentage Inhibition (%)
I	Control	1.4	1.4	0	-
II	Formalin induced	1.5	3.2	1.7	-
III	C1-50 mg/kg	1.5	2.0	0.5	66.7
IV	C1-100 mg/kg	1.4	2.1	0.7	50
V	C2-50 mg/kg	1.4	2.0	0.6	57.14
VI	C2-100 mg/kg	1.4	2.1	0.7	50
VII	Indomethacin 10 mg/kg	1.5	1.6	0.1	93.75

The formalin induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs. Formalin induces an edema which mainly depends on the release of substance P, prostanoids, 5-hydroxytryptamine and histamine. C1 and C2 showed significant dose dependent inhibition in paw volume and elicited anti-inflammatory response comparable with indomethacin. Histamine, vascular endothelial growth factor, acetyl choline, estrogen and fluid shear stress activate a mechanism in Hsp90. This activity was performed to investigate whether the test compounds C1 and C2 have anti-inflammatory activity.

Heat shock protein90 (Hsp90) binding to endothelial nitric oxide synthase (eNOS) is an important step in eNOS activation is the primary source of NO in inflammation. Binding of eNOS to Hsp90 has been shown to occur in response to vascular endothelial growth factor (VEGF), histamine, and eNOS activity is increased in a dose-dependent manner in the presence of Hsp90. Interaction between Hsp90 and eNOS enhances the nitric oxide production leads to increased vascular permeability causing inflammation. Administration of two test compounds C1 and C2 toformalin induced rats have shown a dose dependent inhibition of the paw volume and this might be due to blocking the eNOS through Hsp90 inhibition [14].

CONCLUSION

The anti-Parkinson's activity was performed using reserpine induced Parkinson's model and haloperidol induced Parkinson's model, The two test compounds C1 and C2 have shown significant dose dependant reduction in symptoms like muscular rigidity, Palpebral ptosis, tremor, bradykinesia, righting reflex, catalepsy, actophotometer, swim test, locomotor behaviour indicating their Antiparkinson's activity. The test compounds C1 and C2 produced significant inhibition of paw volume in both carrageenan and formalin induced paw edema models indicating their reduction in paw volume of both test compounds in dose dependent manner. Histopathological findings showed that C1 & C2 treated animals had decreased infiltration of neutrophils, reduced intracellular space, increased density of cells, and regained normal architecture and moderate necrosis in striatum region of brain. From the above, it was concluded that the two test compounds C1 and C2 possess anti-inflammatory and anti-Parkinson's activities. In reducing the inflammation, among two test compounds C1 & C2, C1 was more potent thanC2. But test compound C2 has shown much potent anti-Parkinson's activity over C1.

REFERENCES:

1. Lau LMde and Breteler MM. Epidemiology of Parkinson's disease. *The Lancet Neurology* **2006**;5(6):525–535.
2. Kalia LV and Lang AE. Parkinson's disease. *The Lancet* **2015**;386(9996):896–912.
3. Polymeropoulos MH, Lavedan C, Leroy E. Mutation in the *α*-synuclein gene identified in families with Parkinson's disease. *Science* **1997**;276(5321):2045–2047.
4. Singleton AB, Farrer M, Johnson J. *α*-synuclein locus triplication causes Parkinson's disease. *Science* **2003**;302(5646):841.
5. Andrew L, Clare EW, McArthur K, Nguyen T and Donald A. Hsp90 inhibition supress's lipopolysaccharide induced lung inflammation *In-Vivo.* **2015**:1/16-16/16.
6. McGeer PL, Itagaki S, Akiyama H and McGeer EG. Rate of cell death in Parkinsonism indicates active neuropathological process. Annals of Neurology **1988a**;24:574-576.
7. Kosloski LM, Ha DM, Hutter JA, Stone DK, Pichler MR, Reynolds AD, Gendelman HE, Mosley RL. Adaptive immune regulation of glial homeostasis as an immunization strategy for neurodegenerative diseases. J Neurochem **2010**;114:1261–1276.
8. Jose L and Rodrigo B. Behavioral analysis of the reserpine induced motor changes in a parkinson's mouse model. Revistaneurociencias **2011**;11:49-61.
9. Rajaram C, Ravindra Reddy K and Chandra Shekar. Neuroprotective activity of tephrosia purpura against haloperidol induced Parkinson`s disease model. *Pharmacologia* **2015**;6(4):125-130.
10. Neha P, Suganthi V and Gowri S. Evalution of anti-inflammatory activity in ethanolic extract of *coriandrum sativum* using carrageenan induced paw oedema in albino rats. *Der Pharma Chemica* **2013**;5(2):139-143.
11. Subhashini N, Purnima S, Amutha J, Aiswarya Devi, Thanga A and Lavanya. Anti-inflammatory activity of *Erythrinastricta*roxb in albino rats. *Int J PharmaTech Res* **2011**;3:1014-1018.
12. Josh LC, Rodrigo and Bolanos. Behavioural analysis of the reserpine induced motor changes in a Parkinsonian mouse model. **2011**;11:49-61.
13. Kalia SK, Kalia LV and McLean PJ. Molecular chaperones as rational drug targets for parkinson's disease therapeutics. CNS Neurologic Disorders Drug Targets **2010**;9(6):741-753.
14. Mariarosaria B, Fiorentina R, Carla C and William C. Geladnamycin an inhibitor of heat shock protein 90 mediated signal transduction has antiinflammatory effects and interacts with glucocorticoids receptor *in vivo*. *Brit J Pharmacol* **2000**;131:13-16.
15. Oday O, Rehman U, Tahir M, Rehan Khan, Abdul Quaiyoon, Abdul Lateef, Farrah Ali and Sultana S. Amelioration of 1,2 dimethylhydrazine (DMH) induced colon oxidative stress, Inflammation and tumor promotion response by tannic acid in wistar rats. *Asian Pacific J Cancer Preventi* **2012**;13(9):4393-4402.

How to cite this article:

M. Ganga Raju et al. MOLECULAR CHAPERONES AS THERAPEUTIC TARGETS FOR PARKINSONISM AND INFLAMMATION. J Pharm Res 2017;6(Suppl 2):18-24.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil