Design, Synthesis, and Neuroprotective Effects of Ethyl 2-'amino thiazole-4-carboxylate derivatives against 6-Hydroxydopamine (6-OHDA) induced Parkinsonism

Rajnish Kumar Malik[1], Tanveer Naved[1]*, Vikramdeep Monga[2*], Amit Kumar Sharma[1]

[1]*Amity Institute of Pharmacy, Amity University, Noida Campus, Uttar Pradesh, India-201313*

[2]*Department of Pharmaceutical Sciences and Natural Products, Central University of Punjab, Ghudda, Bathinda, Punjab, India-151401*

**Corresponding Author(s) E-mail address: tnaved@amity.edu (Dr. Tanveer Naved); vikramdeepmonga@gmail.com (Dr. Vikramdeep Monga)*

Manuscript Received: 19 Jul 2022 Revised: 06 Nov 2022 Accepted: 14 Dec 2022

Abstract

Parkinson disease is a neurodegenerative disorder characterised by the cardinal symptoms of stiffness, resting tremor, slowness (bradykinesia) and reduction of movement (hypokinesia). Involvement of oxidative damage has been reported in the pathophysiology of Parkinson disease and its related complications. The purpose of the present work was to study the effect of two novel compounds on 6-ODHA induced Parkinson disease. The effect was evaluated by assessing various behavioral and biochemical parameters (lipid peroxidation, and reduced glutathione) in brain tissue. Levodopa was used as standard anti-parkinsonian drug. 6-OHDA significantly causes tremor, rigidity, akinesia and oxidative damage which was significantly reversed by administration of novel synthesized compounds i.e., SH-4 & SH-9 when compared to 6-OHDA group. So, the results indicated the protective effect of synthesized compounds against PD.

Keywords: Parkinson disease, 6-ODHA, Levodopa, anti-parkinsonism activity, Ethyl 2-((5-(furan-2-ylmethylene)-4-oxothiazolidin-2-ylidene) amino)-thiazole-4-carboxylate (SH-4), N'-(4-(benzyloxy)-3-methoxybenzylidene)-2-acetamidothiazole-4-carbohydrazide (SH-9)

DOI: 10.48047/ecb/2023.12.3.193

1. Introduction

James Parkinson identified Parkinson disease (PD) as a complex, progressive neurodegenerative disease in his 1817 publication, "Essay on the Shaking Palsy." Lewy bodies, are cytoplasmic inclusions that contain insoluble alpha-synuclein aggregates, and are related to the loss of dopaminergic neurons in the substantia nigra pars compacta (SN), a region of the midbrain, which is the pathological definition of Parkinson's disease (PD) [1]. But PD also affects non-dopaminergic neurons and is characterized by more widespread disease in other parts of the brain. The clinical diagnosis of Parkinson's disease (PD) is primarily based on

motor features, including a slowly progressing asymmetric resting tremor, cogwheel rigidity, and bradykinesia [2].

However, non-motor features, such as anosmia, constipation, depression, and Rapid Eye Movement (REM), sleep behaviour disorder, can appear years before motor deficits. Additional nonmotor symptoms, including as autonomic dysfunction, pain, and cognitive impairment, may manifest in the later stages of the disease [3]. Years before PD begins, there is a prodromal phase during which many individuals experience gastrointestinal problems. These signs provide credence to Braak's theory and paradigm, according to which pathogenic synuclein spreads from the gut to the brain [4].

Notably, the etiology of Parkinson's disease is significantly influenced by immunological responses [5]. Microglia can induce innate immune responses that result in neuronal degeneration and the development of disease [6]. Additionally, T cells migrate into PD patient's brains and participate in adaptive immune responses. Interestingly, synuclein interacts with microglia and T cells directly to influence both innate and adaptive immune responses [7].

The nigrostriatal circuit has traditionally been targeted with toxins or genetic therapies that mimic motor impairments to create the recent PD animal models. 6-hydroxydopamine (6-OHDA), which was first discovered more than 50 years ago, is still often used to imitate Parkinson's disease [8]. Due to its inability to pass the blood-brain barrier, this substance has not been linked to Parkinson's disease as an aetiologic agent. Because of this, 6-OHDA needs to be administered directly into the substantia nigra's dopamine (DA) cell bodies. Both the DA and noradrenergic (NA) systems in the brain are damaged by 6-OHDA injections into the lateral ventricles. The buildup of 6-OHDA by DA neurons through their high-affinity uptake system is what gives 6-OHDA its selectivity [9].

When 6-OHDA is administered into the striatum, it causes slow, prolonged retrograde degeneration, which leads to a more progressive and stable loss of DA neurons. A progressive and less severe lesion is more pertinent in the context of PD, hence this route of administration has certain advantages over the other locations [10]. However, the substantial mortality rate seen in mice with bilateral lesions can be avoided by unilaterally administering 6-OHDA, permitting the use of dosages able to generate motor phenotype without endangering the welfare of the animals [11]. In the current investigation, we used the 6-OHDA mouse model and carried out a thorough behavioral and biochemical characterization for Parkinson's disease.

2.0 Material and Method:

2.1 Material

In the investigation analytical grade chemicals were used. Chemicals were supplied by Sigma Aldrich Chemicals Private Limited, Bangalore, India.

2.2 Method:

2.2.1 Synthesis of Ethyl 2-((5-(furan-2-ylmethylene)-4-oxothiazolidin-2-ylidene) amino)-thiazole-4-carboxylate (SH-4)

Chloroacetyl chloride (0.015 mol) was added dropwise into the precursor solution of ethyl 2-aminothiazole-4-carboxylate (0.01 mol) in dry toluene (50 mL) while it was being stirred at room temperature. The reaction mixture was heated under reflux for 4 to 6 hours. The solvent was then evaporated *in vaccuo*, the resultant residue was triturated with water, filtered, dried, and then recrystallized from ethanol to produce good yield and pure ethyl 2-[2-chloroacetamido]-thiazole-4-carboxylate. Absolute ethanol was used to reflux ethyl 2-[2-chloroacetamido]-thiazole-4-carboxylate (0.005 moL) with ammonium thiocyanate (0.010 moL) for 3–4 hours. Precoated TLC plates were used to monitor the reactions progress. After the reaction is finished, keep the mixture overnight. In order to get the ethyl 2-(4-oxothiazolidin-2-ylideneamino)-thiazole-4-carboxylate in good yield and purity, the solvent was then evaporated in-vaccuo, the residue obtained was triturated with water, filtered, dried, and then recrystallized from absolute ethanol. A mixture of ethyl 2-[(Z)-(4-oxo-1,3-thiazolidin-2-ylidene) amino]-1,3-thiazole-4-carboxylate (20 mmol) and fuarn-2-carboxaldehyde (20 mmol) was suspended in absolute ethanol and to this catalytic amount of piperidine (2 mmol) was added. For 4 hours, the mixture was refluxed at 80°C while being stirred. The development of the reaction was monitored by using percolated TLC plates. After the reaction was completed, the mixture was poured into ice cold water and acidified with glacial acetic acid to a pH of 3–4 to yield crude solid. To obtain the pure product, i.e**.,** ethyl 2-((5-(furan-2-ylmethylene)-4-oxothiazolidin-2-ylidene) amino)-thiazole-4-carboxylate (SH-4), the crude product was filtered, extensively washed with water and recrystallized from methanol. Ethyl 2-aminothiazole-4-carboxylate (2 g) and glacial acetic acid (2 mL) were refluxed for about 30 minutes in the presence of acetic acid anhydride (6 mL). Filtered, extensively rinsed in water to remove any leftover glacial acetic acid, and then recrystallized from ethanol to provide an excellent yield of Ethyl-2-acetamido thiazole-4-carboxylate (83%).

2.2.2 Synthesis of N'-(4-(benzyloxy)-3-methoxybenzylidene)-2-acetamidothiazole-4-carbohydrazide (SH-9)

In the presence of absolute ethanol, the 2-acetamido thiazole-4-carboxylate (0.019 mol) and hydrazine hydrate (0.056 mol) were refluxed for 10 hours. To eliminate excess hydrazine hydrate, the obtained white solid was washed with aqueous ethanol before being recrystallized from the ethanol to get the pure product. Equimolar amounts of 2-acetamido thiazole-4-carbohydrazide were refluxed with substituted benzaldehyde in the presence of absolute ethanol and a few drops of glacial acetic acid for 14 hours. The resulting liquid was then put into ice water, and the solid that was produced was then filtered out and thoroughly cleaned with water. By recrystallizing the crude product from ethanol, the desired yield and purity of the title chemical, N'-(4-(benzyloxy)-3-methoxybenzylidene)-2-acetamidothiazole-4-carbohydrazide (SH-9), were obtained.

2.3 Experimental design

2.3.1 Animals

Swiss albino mice, 4 weeks old and weighing 18-20 g were housed in groups of 2 per cage, in the standard conditions of the animal room 22°C room temperature, 55% relative humidity, food and water available ad libitum. All experiments were performed as per CPCSEA guidelines. Animals were divided into five groups of 6 mice in each group. Group I served as sham operated animals and received normal saline (10 mL/kg, p.o.); Groups II to V were induced with parkinsonism by 6-OHDA as follows: Group II served as a 6-OHDA control and received normal saline (10 mL/kg), Group III served as a L DOPA (100 mg/kg, p.o.), and Groups IV to V served as a test drug (Compound 1 (SH-4) and Compound 2 (SH-9), 100 mg/kg, p.o., resp.). The treatment of animals was started after 48 h of induction with 6-OHDA according to their respective group once a day for 55 days.

2.3.2 Induction of Parkinsonism by 6-OHDA

Mice were intraperitoneally (i.p.) anesthetized with ketamine-medetomidine (75 mg/kg; 1 mg/kg) diluted in 0.9% NaCl. Once anesthetized, animals were positioned on a stereotaxic frame (Stoelting, Kiel, WI, USA), and unilateral lesions were achieved by injection of 6-OHDA hydrochloride (Sigma, Germany) in the right hemisphere using a 10 μL Hamilton syringe (DDBioLaB, Barcelona, Spain). The neurotoxin was used at a concentration of 6 μg/μL (calculated from free base weight) dissolved in a solution of 0.9% NaCl in 0.2 mg/mL ascorbic acid. 6-OHDA was administrated into the dorsolateral striatum (2×1.5 μL) at a rate of 1 μl/min,

at the following stereotaxic coordinates (relative to bregma, in mm): (i) AP = +1.0, ML = −2.1, DV = −2.9; (ii) AP = +0.3, ML = −2.3, DV = −2.9. After each injection, the needle was left in place there for 5 min for diffusion and to avoid any backflow. The control group was injected in the same conditions with the neurotoxin vehicle. Anesthesia was reversed using Atipamezole (1 mg/mL). Animals were randomly assigned into experimental groups [12].

After surgery, a post-operative period of 14 days was necessary to allow the degeneration of DA-containing neurons (corresponding to the delay of action of 5,7-DHT, another neurotoxin acting on 5-HT system with similar mechanism of action). Every animal was housed individually during 48 h post-surgery; then they were housed in their respective groups.

2.4 Behavioural assessment

2.4.1 Locomotor Activity

The spontaneous locomotor activity was monitored using digital Actophotometer (Hicon instrument, India) equipped with infrared sensitive photocells. The apparatus was placed in a darkened, light and sound attenuated, and ventilated testing room. Each interruption of a beam on the x or y axis generated an electric impulse, which was presented on a digital counter. Each animal was observed over a period of 5 min on days 15, 20, 25, 30, 35, 40, 45, 50, and 55 following 6-OHDA administration and values were expressed as counts per 5 min.

2.4.2 Rotarod

All animals were evaluated for grip strength by using the rotarod. The rotarod test is widely used in rodents to assess their "minimal neurological deficit" such as motor function and coordination. Each rat was given a prior training session before initialization of therapy to acclimatize them on a rotarod apparatus (EIE instrument, India). Animal was placed on the rotating rod with a diameter of 7 cm (speed 25 rpm). Each mice was subjected to three separate trials at 5 min interval on days 15, 20, 25, 30, 35, 40, 45, 50, and 55 following 6-OHDA administration and cut-off time (180 s) was maintained throughout the experiment. The average results were recorded as fall of time.

2.4.3 Forced swim test

The forced swim test was utilized to assess depressive-like behaviour. Mice were kept in a water-filled cylinder (24°C), for 5 min. The test was recorded via a video camera and latency

to stop and immobility duration were calculated. Depressive-like state was defined as an increase in the duration of immobility and decrease in latency to immobility.

2.4.4 Sucrose preference test

Anhedonia was assessed using the sucrose preference test. The test consisted of depriving animals food and water for 18 h and afterwards presenting two pre-weighted (separate) bottles, one containing 3% sucrose solution and a second containing tap water, during 1 h. Reduced sucrose preference is suggestive of anhedonia and thus of depression-like behavior.

2.4.5 Open field

The open field was used to assess locomotor behavior. The mice were placed in the centre of a square (43.2 cm × 43.2 cm) arena (ENV-515, Med Associates Inc.) and allowed to explore the arena for 5 min. With the help of the tracking software (SOF-811, Med Associates Inc.) total distance travelled, time in rest, number of rearings and ambulatory episodes were recorded. The arena was cleaned with 10% ethanol between each animal [13].

2.5 Dissection and Homogenization

After the treatment period, animals were scarified by decapitation under mild anesthesia. The brains were immediately removed, forebrain was dissected out, and cerebellum was discarded. Brains were put on ice and rinsed in ice-cold isotonic saline to remove blood. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 minutes and aliquots of supernatant obtained were used for biochemical estimation.

2.5.1 Biochemical Estimation

2.5.1.1 Malondialdehyde (MDA) Level

The amount of malondialdehyde was used as an indirect measure of lipid peroxidation and was determined by reaction with thiobarbituric acid (TBA) [14]. Briefly, 1 mL of aliquots of supernatant was placed in test tubes and added to 3 mL of TBA reagent: TBA 0.38% (w/w), 0.25 M hydrochloric acid (HCl), and trichloroacetic acid (TCA 15%). The solution was shaken and placed for 15 min, followed by cooling in an ice bath. After cooling, solution was centrifuged to 3500 g for 10 min. The upper layer was collected and assessed with a spectrophotometer at 532 nm. All determinations were made in triplicate. Results were expressed as nanomoles per mg of protein.

2.5.1.2 Superoxide Dismutase (SOD) Level

SOD activity was determined according to the method described by Beyer and Fridovich in 1987 [15]. 0.1 mL of supernatant was mixed with 0.1 mL EDTA (1×10^{-4} M), 0.5 mL of carbonate buffer (pH 9.7), and 1 mL of epinephrine (1 mM). The optical density of formed adrenochrome was read at 480 nm for 3 min on spectrophotometer. The enzyme activity was expressed in terms of U/min/mg. One unit of enzyme activity is defined as the concentration required for the inhibition of the chromogen production by 50% in one minute under the defined assay conditions.

2.5.1.3 Catalase (CAT) Level

The catalase activity was assessed by the method of Aebi in 1974 [16]. The assay mixture consists of 0.05 mL of supernatant of tissue homogenate (10%) and 1.95 mL of 50 mM phosphate buffer (pH 7.0) in 3 mL cuvette. 1 mL of 30 mM hydrogen peroxide (H_2O_2) was added and changes in absorbance were followed for 30s at 240 nm at 15s intervals. The catalase activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.071 mmol $cm^{-}1$) and the activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

2.5.1.4 GSH Level (Reduced Glutathione)

For the estimation of reduced glutathione, the 1 mL of tissue homogenate was precipitated with 1 mL of 10% TCA. To an aliquot of the supernatant, 4 mL of phosphate solution and 0.5 mL of 5,5 - dithiobis-(2-nitrobenzoic acid) (DTNB) reagent were added and absorbance was taken at 412 nm. The values were expressed as nM of reduced glutathione per mg of protein [17].

2.6 Statistical Analysis

All the values were expressed as mean ± SEM. Statistical evaluation of the data was done by one-way ANOVA (between control and drug treatments) followed by Dunnett's t-test for multiple comparisons and two-way ANOVA followed by Bonferroni's multiple comparison test, with the level of significance chosen at $p<0.05$ using Graph-Pad Prism 5 (San Diego, CA) software.

3.0 Results and Discussion

3.1 Synthesis of Novel Compounds

In the present study, Ethyl 2-((5-(furan-2-ylmethylene)-4-oxothiazolidin-2-ylidene) amino)-thiazole-4-carboxylate (SH-4) was synthesised. N'-(4-(benzyloxy)-3-methoxybenzylidene)-2-acetamidothiazole-4-carbohydrazide (SH-9) was also prepared when equimolar quantity of 2-acetamido thiazole-4-carbohydrazide was refluxed with substituted benzaldehyde in the presence of absolute ethanol and few drops of glacial acetic acid for 14 hrs.

3.2 Behavioural assessment

3.2.1 The Effects of SH-4 and SH-9 on 6-OHDA induced Parkinson's Disease in the Locomotor Activity

Total locomotor activity of mice in 6-OHDA treated group was significantly (*$p<0.05$) reduced as compared to vehicle treated group. Oral administration of test compounds (SH-4 & SH-9) showed significant (*$p<0.05$) increase in the locomotor activity from day 20 to 55 as compared to 6-OHDA treated control animals. The locomotor activity on 55th day was reported here. Levodopa (100 mg/kg) significantly (**$p<0.001$) increased locomotor activity (Figure 1).

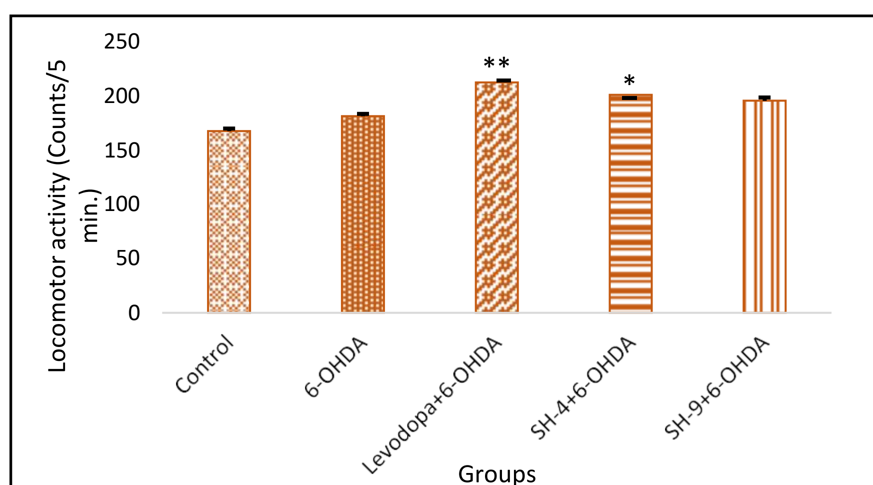

Figure 1: Effect of SH-4 and SH-9 treatment on locomotor activity in 6-ODHA induced PD model. Values are expressed as mean ± SEM; n = 6/group; Levodopa (p<0.001) and SH-4 (*p<0.05) represented significant difference from 6-ODHA group.**

3.2.2 The Effects of SH-4 and SH-9 on 6-OHDA induced Parkinson's Disease in the Rotarod Performance

Treatment with 6-OHDA significantly decreased the fall of time when compared to the vehicle control animals. Chronic oral administration of SH-4 and SH-9 significantly (*$p<0.05$) increased the fall of time when compared to 6-OHDA group from day 15 to day 55. Levodopa

(100 mg/kg) significantly (***p*< 0.001) increased the fall of time as compared to 6-OHDA group (Figure 2).

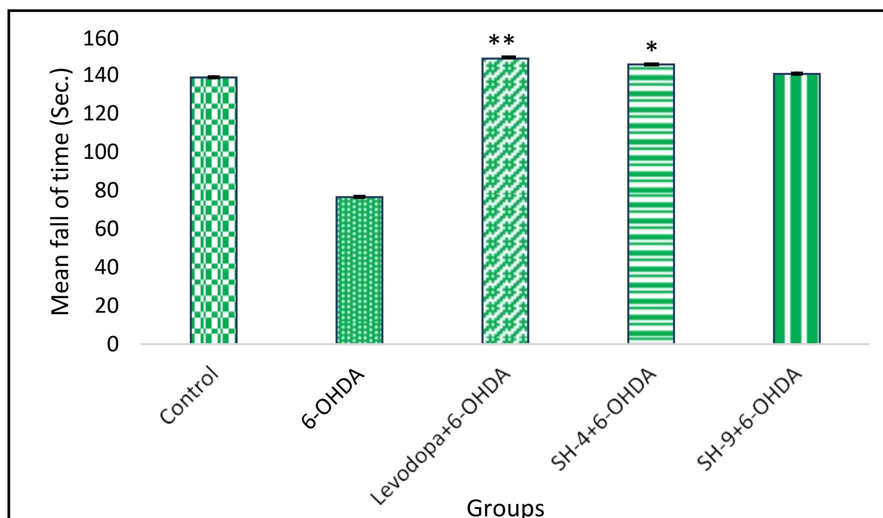

Figure 2: Effect of SH-4 and SH-9 treatment on Rotarod activity in 6-ODHA induced PD model. Values are expressed as mean ± SEM; n = 6/group; Levodopa (p<0.001) and SH-4 (*p<0.05) represented significant difference from 6-ODHA group.**

3.2.3 The Effects of SH-4 and SH-9 on 6-OHDA induced Parkinson's Disease in the Forced Swim Test

The amount of time spent immobile was significantly reduced by SH-4 & SH-9 compounds (Figure 3). Levodopa also demonstrated a notable (***p*<0.001) reduction in the time spent immobile. Moreover, the test compounds i.e., SH-4, showed significant decrease (*p<0.05) in a time of immobility closed to Levodopa.

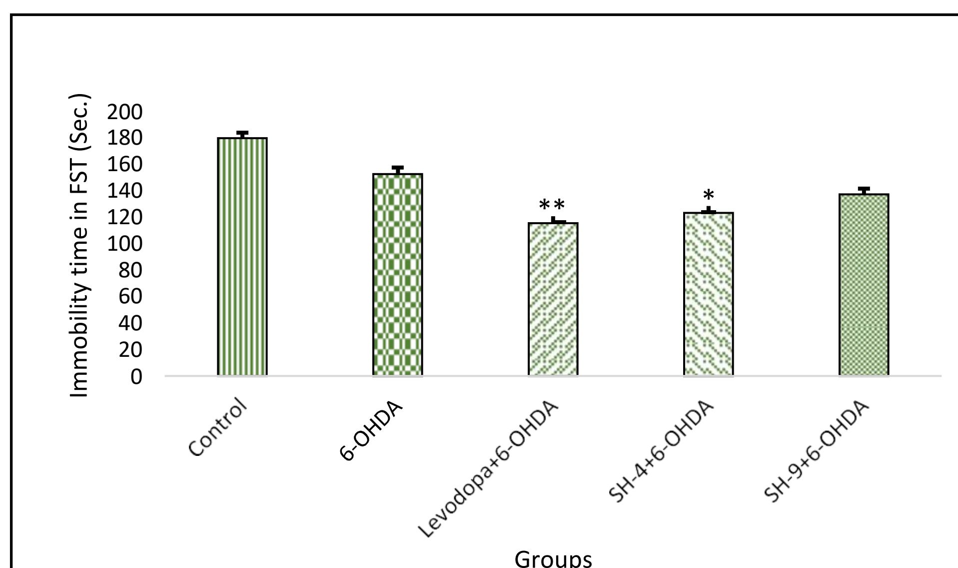

Figure 3: Effect of SH-4 and SH-9 treatment on Immobility time in 6-ODHA induced PD model. Values are expressed as mean ± SEM; n = 6/group. Levodopa (p<0.001) and SH-4 (*p < 0.05) represented significant difference from 6-ODHA group.**

3.2.4 The Effects of SH-4 and SH-9 on 6-OHDA Induced Parkinson's Disease in the Sucrose Preference Test

The results of the sucrose preference test likewise supported those of the 6-OHDA induced anhedonia in mice, selected compounds i.e., SH-4 & SH-9 improved the sucrose preference. The results obtained with SH-4 supported the antiparkinsonian results close to Levodopa. Results of Sucrose preference test were presented in figure 4.

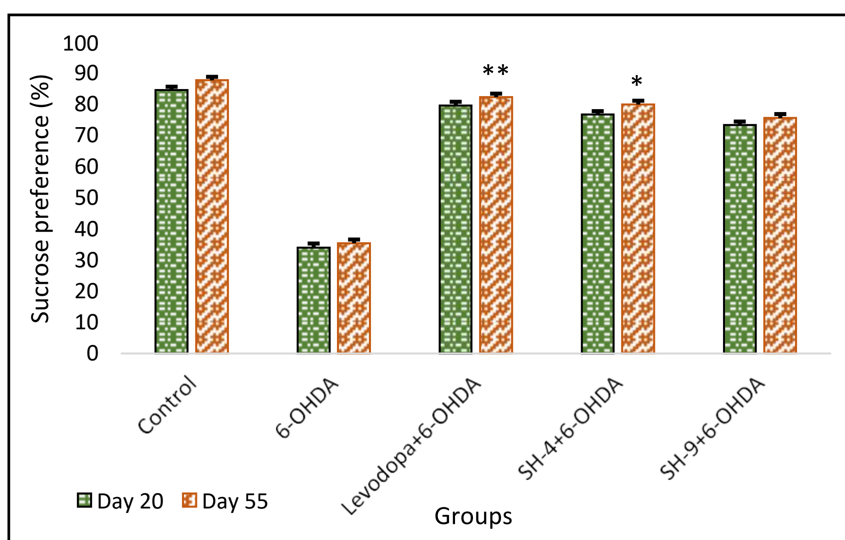

Figure 4: Effect of SH-4 and SH-9 treatment on Sucrose preference test in 6-ODHA induced PD model. Values are expressed as mean ± SEM; n = 6/group; Levodopa group represented significant increase in Sucrose Preference (p<0.001) and SH-4 also represented significant (*p<0.05) difference from 6-ODHA group**

3.2.5 The Effects of SH-4 and SH-9 on 6-OHDA induced Parkinson's Disease in the Open field test

Open field test was used to measure locomotor activity in terms of total distance travelled and average velocity. 6-OHDA treated animals showed significant reduction in total distance travelled as compared to controls. Additionally, average velocity was decreased in 6-OHDA treated mice while SH-4 and SH-9-treated animals showed improvement in velocity. The results were presented in figure 5a and 5b.

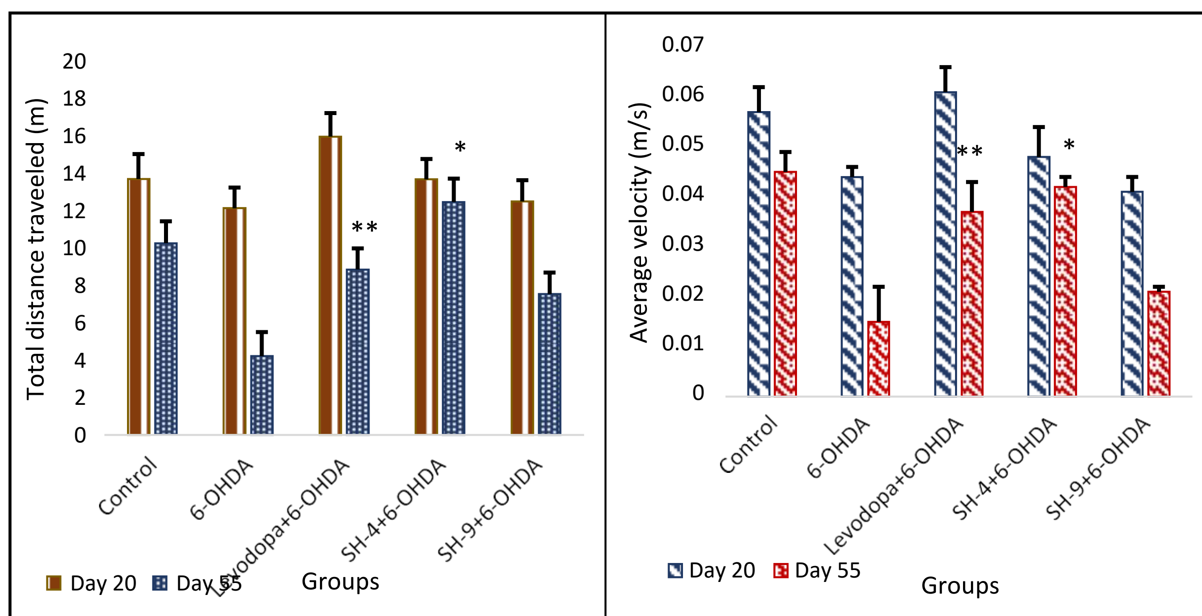

Figure 5: Effect of SH-4 & SH-9 treatment on motor functions assessed using Open field test in 6-ODHA induced PD model. Total distance travelled (A) and Average velocity (B). Values are expressed as mean ± SEM; n = 6/group; A significant difference was seen in Levodopa group (p<0.001) and SH-4 (*p<0.05) group when compared with 6-ODHA group**

3.3 Biochemical Estimation

The effects of SH-4 and SH-9 on MDA, CAT, SOD, and GSH level was studied. Administration of 6-OHDA resulted in significant changes in biochemical parameters when compared to the vehicle control animals. The inoculation of 6-OHDA induced oxidative stress, as indicated by increased MDA level, and decreased CAT, SOD, and GSH levels when compared to vehicle control animals. The treatment with SH-4 showed significant (*$p<0.05$) decrease in MDA level compared to 6-OHDA treated mice. Similarly, daily administration of SH-4 attenuated the increase in SOD and CAT activity with 6-OHDA treated group. Pretreatment with SH-4 significantly (*$p<0.05$) increased GSH levels in the brain as compared to 6-OHDA treated animals, thus preventing the reduction in GSH induced by 6-OHDA (Figure 6). Similarly, the SH-9 showed reduction in MDA level and upgradation of SOD, CAT and GSH level but less significantly than SH-4 group. Moreover, significant results were produced by Levodopa (**p<0.001) treated group.

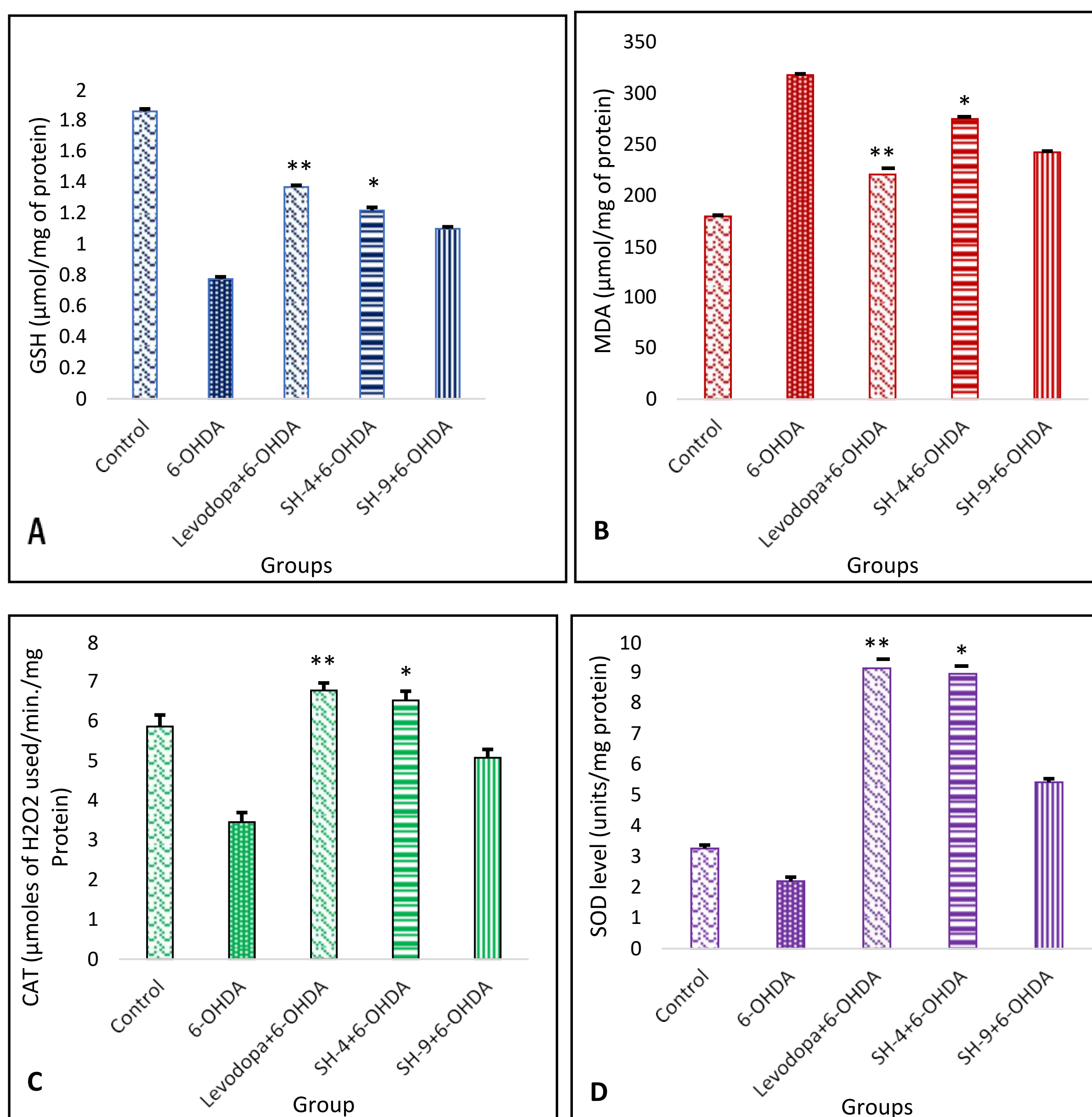

Figure 6: Effect of SH-4 & SH-9 treatment on biochemical parameters i.e., reduced GSH level (A), MDA level (B), CAT level (C) and SOD level (D) assessed in 6-ODHA induced PD model. Values are expressed as mean ± SEM; n = 6/group; A significant difference was seen in Levodopa group (p<0.001) and SH-4 group (*p<0.05) when compared with 6-ODHA group**

4.0 Discussion

Parkinson's disease is a chronic neurodegenerative disorder characterized by loss of dopamine neurons of the SNpc. The pathogenesis of PD includes oxidative stress, protein accumulation like a-synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitotoxicity. Among all, oxidative stress is a crucial pathological mechanism for PD. SNpc is more vulnerable to reactive oxygen species as it contains more amount of dopamine. In the present study, the effect

of two novel synthesized compounds i.e., SH-4 & SH-9 was studied in neurotoxin (6-OHDA) induced Parkinson disease in experimental animals.

In the present study, 6-OHDA administration to mice caused a significant decrease in locomotor activity and muscle activity. Lack of motor coordination and maintenance of normal limb posture has been reported in PD condition. The evaluated data suggested damage to the dopaminergic neurons and progression of Parkinson's disease like behavioral abnormalities in mice exposed to 6-OHDA. Pretreatment of mice with SH-4 & SH-9 exhibited significant increase in locomotor activity and increase in muscle activity and thus could be proved with possible action on CNS.

Oxidative stress generated as a result of mitochondrial dysfunction particularly mitochondrial complex-1 impairment plays an important role in the pathogenesis of PD. The oxidative stress was measured through determination of levels of malondialdehyde, catalase, superoxide dismutase, and reduced glutathione in the brain tissue. 6-OHDA generates an increase in the production of hydrogen peroxide and free radicals [18-19]. The resulting ROS production from 6-OHDA breakdown leads to lipid peroxidation, protein denaturation, and increases in glutathione, which are found in PD patients [20]. Lipid peroxidation, a sensitive marker of oxidative stress, was estimated by measuring the levels of thiobarbituric acid. Superoxide dismutase (known as SOD) is an enzyme which acts as a catalyst in the process of dismutation of superoxide into nonreactive oxygen species and hydrogen peroxide. 6-OHDA treated control group showed a decrease in the level of SOD in the brain of animals, thus indicative of production of oxidative stress.

Thus, the 6-OHDA group showed a significant increase in the levels of thiobarbituric acid (which is an indication of extent of lipid peroxidation) and decrease in the levels of SOD and GSH in the brain as compared to the vehicle treated control animals. All these increased the oxidative stress in the brain of animals treated with 6-OHDA. Pretreatment with synthesized compounds i.e., SH-4 & SH-9 resulted in a decrease in MDA level and increase in the levels of SOD, catalase, and GSH, indicating its antioxidant and anti-parkinsonism effects in the brain of 6-OHDA treated animals.

5.0 Conclusion

In view of the above facts, it was concluded that synthesized compounds showed to be an antioxidant and showed a promising effect in animals with Parkinson's disease. However,

further detailed studies with the selected compounds in anti-Parkinson's pharmacology and toxicology is required.

References:

1. Simon DK, Tanner CM, Brundin P. Parkinson disease epidemiology, pathology, genetics, and pathophysiology. Clinics in geriatric medicine. 2020 Feb 1;36(1):1-2.
2. Sung VW, Nicholas AP. Nonmotor symptoms in Parkinson's disease: expanding the view of Parkinson's disease beyond a pure motor, pure dopaminergic problem. Neurologic clinics. 2013 Aug 1;31(3):S1-6.
3. Lee HM, Koh SB. Many faces of Parkinson's disease: non-motor symptoms of Parkinson's disease. Journal of movement disorders. 2015 May;8(2):92.
4. Zhu B, Yin D, Zhao H, Zhang L. The immunology of Parkinson's disease. InSeminars in Immunopathology 2022 Sep (Vol. 44, No. 5, pp. 659-672). Berlin/Heidelberg: Springer Berlin Heidelberg.
5. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. Nature Reviews Immunology. 2022 Nov;22(11):657-73.
6. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE. Parkinson disease. Nature reviews Disease primers. 2017 Mar 23;3(1):1-21.
7. Contaldi E, Magistrelli L, Comi C. T lymphocytes in Parkinson's disease. Journal of Parkinson's Disease. 2022 Jan 1;12(s1):S65-74.
8. Mendes-Pinheiro B, Soares-Cunha C, Marote A, Loureiro-Campos E, Campos J, Barata-Antunes S, Monteiro-Fernandes D, Santos D, Duarte-Silva S, Pinto L, José Salgado A. Unilateral intrastriatal 6-hydroxydopamine lesion in mice: a closer look into non-motor phenotype and glial response. International Journal of Molecular Sciences. 2021 Oct 26;22(21):11530.
9. Mokrý J. Experimental models and behavioural tests used in the study of Parkinson's disease. Physiol Res. 1995 Dec;44:143-50.
10. Bové J, Perier C. Neurotoxin-based models of Parkinson's disease. Neuroscience. 2012 Jun 1;211:51-76.
11. Bagga V, Dunnett SB, Fricker RA. The 6-OHDA mouse model of Parkinson's disease–terminal striatal lesions provide a superior measure of neuronal loss and replacement than median forebrain bundle lesions. Behavioural brain research. 2015 Jul 15;288:107-17.

12. Mendes-Pinheiro, B.; Soares-Cunha, C.; Marote, A.; Loureiro-Campos, E.; Campos, J.; Barata-Antunes, S.; Monteiro-Fernandes, D.; Santos, D.; Duarte-Silva, S.; Pinto, L.; et al. Unilateral Intrastriatal 6-Hydroxydopamine Lesion in Mice: A Closer Look into Non-Motor Phenotype and Glial Response. Int. J. Mol. Sci. 2021, 22, 11530. https:// doi.org/10.3390/ijms222111530.
13. Jitendra O. Bhangale and Sanjeev R. Acharya. Anti-Parkinson Activity of Petroleum Ether Extract of Ficus religiosa (L.) Leaves. Advances in Pharmacological Sciences Volume 2016, Article ID 9436106, 9 pages http://dx.doi.org/10.1155/2016/9436106.
14. H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Analytical Biochemistry, vol. 95, no. 2, pp. 351–358, 1979.
15. W. F. Beyer Jr. and I. Fridovich, "Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions," Analytical Biochemistry, vol. 161, no. 2, pp. 559– 566, 1987.
16. H. Aebi, "Catalase in vitro," in Methods in Enzymology, vol. 105, pp. 121–126, Academic Press, New York, NY, USA, 1984.
17. S. K. Srivastava and E. Beutler, "Accurate measurement of oxidized glutathione content of human, rabbit, and rat red blood cells and tissues," Analytical Biochemistry, vol. 25, pp. 70– 76, 1968.
18. G. Cohen and R. E. Heikkila, "The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6- hydroxydopamine, dialuric acid, and related cytotoxic agents," The Journal of Biological Chemistry, vol. 249, no. 8, pp. 2447– 2452, 1974.
19. D. G. Graham, S. M. Tiffany, W. R. Bell Jr., and W. F. Gutknecht, "Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro," Molecular Pharmacology, vol. 14, no. 4, pp. 644–653, 1978.
20. P. Jenner and C. W. Olanow, "Understanding cell death in Parkinson's disease," Annals of Neurology, vol. 44, no. 3, pp. S72– S84, 1998.