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RESEARCH ARTICLE

Ligand and Structure-Based Hybrid Screening for Anti-Parkinson Agents and their Pharmacological Evaluation

Mudita Mishra[1*], Pankaj K. Sonar[1], Avinash C. Tripathi[1], Shailendra K. Saraf[1], Santosh Kumar Verma[2]

ABSTRACT

The behavioral and biochemical antiparkinson effect of 7-hydroxyflavone (7-HF) was evaluated by using virtual screening with an e-pharmacophore and shape-based screening approach, and the compound was screened by using the Sigma Aldrich compound library. Screened hits were filtered based on Lipinski's rule, absorption, distribution,me tabolism,elimination, (software for evaluation) (ADME), and toxicity parameters. The best scoring hit, 7-hydroxy 2 phenyl-4H-chromen-4-one, i.e., 7-HF was selected based on shape similarity (> 0.7), g-score, and conserved interactions. Toxicity assessment of retrieved hits was carried out by Osiris and Lazar programs. This study aims to obtain some potential hits, against various antiparkinson category from reported literature and available online resources, and validate their potency by *in vivo*, *in vitro* methods. Reserpine 5 mg/kg produces Parkinson's like condition by depleting presynaptic catecholamines, particularly dopamine through the process of degranulation of storage vesicles. 7-HF 25, 50, and 100 mg/kg was used as a test compound. Syndopa 275 mg/kg was used as a standard drug. The results demonstrate that treatment with 7-HF improved the total locomotor activity and muscular coordination in the rotarod test. In the open field test, enhanced rearing, grooming duration of mobility, and gripping strength in the chimney test, while a decrease in cataleptic scores in the bar test. 7-HF significantly increases catalase, superoxide dismutase, and reduces glutathione level, while reduced the Malondialdehyde (MDA) level. The total protein concentration was also increased in 7-HF treated groups. The behavioral and biochemical results obtained from this study disclosed a definite neuroprotective role of 7-HF in a dose-dependent manner. It is also clear that 7-HF showed potent and effective antiparkinson activity in a similar way as standard. Interestingly, in behavioral and biochemical studies, 7-HF showed approximately equivalent effects as compared to syndopa.

Keywords: 7-hydroxyflavone, Catecholamine, Parkinson, Reserpine, Superoxide dismutase, Syndopa.

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[1]Babu Banarsi Das Northern India Institute of Technology, Lucknow

[2]Motherhood University, Roorkee

***Corresponding Author:** Mudita Mishra, Assistant Professor, Faculty of Pharmaceutical Sciences, Motherhood University, Email-muditam8@gmail.com

INTRODUCTION

Parkinson disease (PD) is a common, slowly progressive, and neurodegenerative disorder resulting from the degeneration of dopaminergic neurons in the substantia nigra (SN), a region of the brain that controls the movement.[1] It was described by James Parkinson in 1817 as "shaking palsy."[2] The initial symptoms of PD, include tremor at rest, muscular rigidity, bradykinesia, postural abnormalities, and instability.[3] The clinical manifestation of PD occurs when about 50% of nigral dopaminergic neurons and about 70% of straital dopamine fibers are lost.[4] Recently, rivastigmine also useful in PD by inhibiting the breakdown of acetylcholine (ACh), improve regularity in walking, maintaining speed, and balance.[5] Oxidative stress is known to damage lipids, proteins, and DNA, along with decreased superoxide dismutase (SOD), catalase, and glutathione levels. The etiology of PD results from a defect in mitochondrial function, dysregulation of brain iron, inflammatory responses, and abnormalities of energy metabolism.[6] Without treatment, PD progresses over 5 to 10 years to a rigid akinetic state, in which patients are incapable of caring themselves. Brain-derived neurotrophic factor (BDNF) initiates plastic changes and modulation of synaptic activity, having a role in the etiopathogenesis of PD.[7] The PD can be treated with various drugs, including levodopa, carbidopa, orphenadrine, benztropine, selegiline, pergola, and many more, which act by reversing the symptoms, but these drugs possess various side effects, like nausea and vomiting, respiratory disturbances, hallucinations, orange discoloration of saliva and urine, mania, dyskinesia convulsions, etc.[8,9] Neural apoptosis is normally prevented by neuronal growth factors, including nerve growth factor and brain-derived neurotrophic factor. These growth factors regulate the expression of the two gene products *Bax* and *Bcl-2*, *Bax* being proapoptotic and *Bcl-2* being antiapoptotic. Blocking apoptosis by interfering at specific points on these pathways, represents an attractive strategy for developing neuroprotective drugs.[10] Recent studies have suggested some contribution of the glycogen synthase kinase-3 (GSK-3) to the degeneration of dopaminergic neurons.[11] Evidence shows that oxidative damage

impairs ubiquitination and degradation of proteins by proteasomes. This may aid in the aggregation of synuclein, which forms eosinophilic inclusions, known as Lewy bodies, a pathological hallmark of Parkinson's disease.[12] The formation of these Lewy bodies is a key determinant that differentiates this disorder from other neurodegenerative diseases.[13] Researchers have examined neuroinflammation as a risk factor for Parkinson's disease.[14] The SN has the highest density of microglia and a higher number of reactive glial cells, than do patients without the disease.[15] The genes responsible for the familial form of PD are alpha-synuclein, parkin, *DJ-1*, *PINK-1*, *PTN*, and *LRRK2*.[16] Research on coenzyme Q_{10} (antioxidant and mitochondrial energy enhancer) and other antioxidants that could possibly be neuroprotective against the neurodegeneration found in Parkinson's disease.[17]

VIRTUAL SCREENING

Virtual screening is a technique used to identify potential drugs from a large chemical library. Three methods of virtual screening protocol were applied in the current study; shape-based screening, e-pharmacophore based screening, and ligand oriented pharmacophore-based screening.[18] For pharmacophore model-based screening, energetically favorable explicit site matching was required, scoring better than -1-kcal/mol. The workflow includes ligand preparation using LigPrep, filtering using propfilter on QikProp properties or other structural properties, glide docking at the three accuracy levels, high throughput virtual screening (HTVS), standard precision (SP), and extra-precision (XP).[19] Crystal structures of antiparkinson's targets (monoamine oxidase inhibitor, acetylcholinesterase, and catechol-O-methyl transferase inhibitor) in complex with their co-crystal ligands, i.e., inhibitors, having a resolution of less than 2.5 A°, and R value less than 0.25, were collected from research collaboratory for structural Bioinformatics (RCSB)[20] Protein Data Bank (PDB), and prepared using the protein preparation wizard, located in Maestro software package.[21]

Shape-Based Screening

Some antiparkinson agents from different pharmacological categories and with a good range of biological activity were selected from literature, as reference molecules for shape-based screening, reference molecules Apigenin, Luteolin, Pramipexole,Rasagaline, Tolcapone, 7-Nitroindazole (Figure 1).[22-26]

Energy-optimized structure-based pharmacophores, i.e., e-pharmacophores were generated through docking, post-processing, and e-pharmacophore option situated in the scripts menu bar of the Maestro software package. For e-pharmacophore hypothesis generation, protein complex was selected based on root mean square deviation (RMSD) and re-docking score. The e-pharmacophore model of the selected protein-ligand complex of each antiparkinson target was generated and screened by a chemical database (ASINEX). For shape-based screening, it was performed by pharmacophore type and atom type volume scoring, while in atom type screening, hits having shape similarity above 0.7 were considered for further toxicity evaluation studies. In pharmacophore type, hits having shape similarity above 0.5 were considered (Figure 2).[27]

Ranking of Hits

Hits obtained from shape-based, as well as, e-pharmacophore-based screening were ranked the basis of shape similarity/fitness score, XP g-score, and conserved interactions (compared to reference ligand).

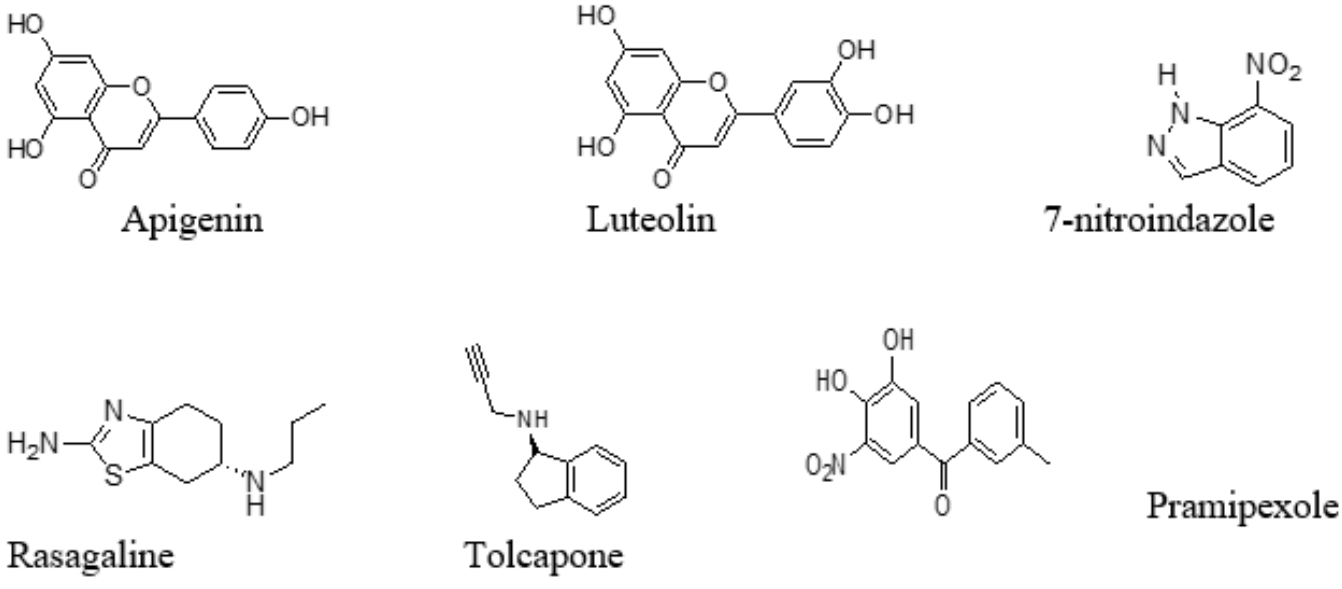

Figure 1: Reference molecule for shape based screening

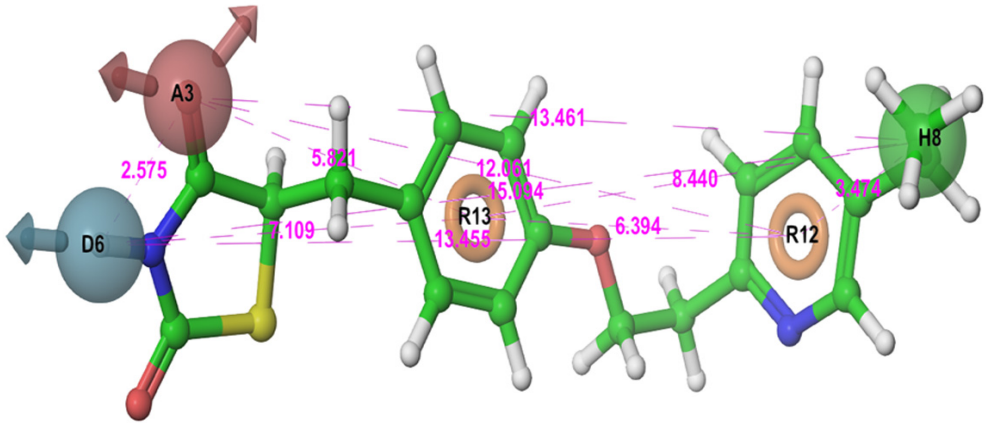

Figure 2: MAOB-4A79; e-pharmacophore models generated by using various protein-ligand complexes against different antiparkinson targets

In silico Toxicity Prediction

Sorted hits were then subjected for toxicity prediction from online available toxicity prediction software, Osiris, and Lazar programs. Finally, the most potential candidate, 1-[4-(4-fluoro-benzyloxy-phenyl]-pyrolidine-3-carboxyllic acid amide, was obtained from the ASINEX compound database was selected for biological evaluations, based on its *in silico* performance. Detail of the toxicity prediction summary was given in the additional file.

Since top scoring hit (1-[4-(4-fluoro-benzyloxy-phenyl]-pyrolidine-3-carboxyllic acid amide, obtained from the ASINEX database were found to be very costly, therefore, on a second priority basis, some more hits were screened from Sigma Compound Library, which is commercially available at a reasonable cost. Shape-based screening of Sigma Aldrich Compound Library against selected query ***mol***ecules was performed, and the obtained hits were filtered based on Lipinski's Rule, ADME, and toxicity parameters was done. The final selection of the screened hits was done taking shape similarity (> 0.7), *g*-score, and conserved interactions. The best scoring hit 7-hydroxy 2 phenyl-4H-chromen-4-one, i.e., 7-HF were procured to study biological activity.[28] Detail summary of hits obtained after screening with Sigma Aldrich compound library attached in additional file.

MATERIAL AND METHODS

7-HF (7-hydroxy 2 phenyl-4H-chromen-4-one) was procured from Tokyo Chemicals Industry (TCI). Reserpine was purchased from SD Fine-Chemicals Limited SDFCL (India). Bovine serum albumin, 5, 5′-dithiobisnitro benzoic acid (DTNB), and pyrogallol were procured from HiMedia Laboratories. Biuret reagent was purchased from Rankem. All other chemicals and reagents used in the experiments were of analytical grade.

Animals

Adult male Swiss albino mice (20–25 grams) were procured from the Animal House, Faculty of Pharmacy, BBDNIIT, Lucknow, UP. All the animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee. The protocol was approved by the Institutional Animal Ethics Committee.

Animals were randomly divided into six groups, containing six animals in each group (n = 6). Reserpine 5 mg/kg body weight (intraperitoneally) was given to all groups, except negative control as Parkinson's inducing agent. The dosing of the test and standard compound was performed by the oral route. The groups were as follows:

- Group I-Vehicle treated (1% **cm**C).
- Group II-Negative control (water).
- Group III-Test substance 25 mg/kg body weight.
- Group IV-Test substance 50 mg/kg body weight.
- Group V-Test substance 100 mg/kg body weight.
- Group VI-Standard drug (syndopa) 275 mg/kg body weight.

Reserpine induces the depletion of central catecholamine stores. Animals were injected intraperitoneally with 5 mg/kg body weight of reserpine and tested 24 hours later. The test compounds were given orally, thirty minutes before observation. The animals were placed singly onto the floor of the slab. Horizontal movements were recorded for 10 minutes.[29]

Rotarod Test

The test is used to evaluate the activity of drugs interfering with motor coordination. Only those animals were used for this test, who demonstrated their ability to remain on the revolving rod for at least 1-minute, and they were trained to stay on an

accelerating rotarod that rotates at a rate of 15 revolutions/minute.[30]

Open Field Test

The open-field consisted of a square arena (40 × 40 cm) and a wall (35 cm high). The square arena was divided into 16 sub-squares. The test was initiated by placing the mouse at the center of the arena. The behavior of the mouse was then observed for 5 minutes. After each test, the apparatus was thoroughly cleaned with a cotton pad wetted with 70% ethanol. The number of line crossings (crossing the boundaries of the square with both forepaws), rearings (standing on its hind legs), grooming (rubbing the body with paws or mouth, and rubbing the head with paws), and duration of immobility were measured.[31]

Catalepsy

Catalepsy activity was measured by the bar test. In the bar test, the mice were placed with both the front paws on a horizontal bar. The bar was 3 cm above, and parallel to the base in half rearing position. The amount of time spent by a mouse with at least one forepaw on the bar was determined.

Total Locomotor Activity

Actophotometer was used to measure locomotor activity. Animals were initially allowed to get acclimatized in the chamber for a period of 2 minutes, and then their locomotor activity was monitored for the next 5 minutes. Total locomotor activity was calculated as a mean of photo beam counts per 5 min/animal.[32]

Chimney Test

Each mouse was introduced into a transparent plastic tube (3 cm in inner diameter, 25 cm in length) with head forward. When the mouse reached the other end of the tube (gentle push if necessary), the tube was moved to a vertical position. The mouse tried to climb backward. The time required for the mouse to climb backward out of the cylinder was noted, and cut off time was 240 seconds.[33]

Biochemical Test

Estimation of Biochemical Parameters of Oxidative Stress

The biochemical parameters of oxidative stress were estimated in all the groups after the completion of behavioral studies. These estimations were done in the whole-brain homogenate.

Brain Collection and Preparation of Homogenate

Mice were cervically dislocated under ether anesthesia. The skull was cut, open, and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples was prepared by using a homogenizer to estimate biochemical markers.

Determination of Catalase Activity

The catalase activity was determined by the method of Oyedemi *et al.* and authors method used for catalase activity estimation. In brief, the reaction mixture consisted of 0.4 mL of hydrogen peroxide (0.2 M), 1 mL of 0.01 M phosphate buffer (pH 7), and 0.1 mL of brain homogenate (10% w/v). The reaction of the mixture was stopped by adding 2 mL of dichromate-acetic acid reagent (5% $K_2C_{r2}O_7$ prepared in glacial acetic acid). The changes in the absorbance were measured at 620 nm and recorded. Percentage inhibition of catalase was calculated using the equation given below.

% catalase inhibition = (normal activity - inhibited activity)/(normal activity) × 100

Determination of Reduced Glutathione Activity (GSH)

In this method, an aliquot of 1-mL of the supernatant of homogenate was treated with 0.5 mL of Ellman's reagent (19.8 mg of DTNB in 100 mL of 0.1% sodium nitrate) and 3 mL of phosphate buffer (0.2 M, pH 8). The absorbance was measured at 412 nm. The percentage inhibition of GSH was calculated using the following equation.

% reduced glutathione inhibition = $(A_o - A_1)/A_o \times 100$

Where, A_o is the absorbance of the control and A_1 is the absorbance of the sample.

Estimation of Lipid Peroxidation

Lipid peroxidation in the brain was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS). In brief, 0.1 mL brain homogenate (10% w/v) was treated with 2 mL of the mixture of 1:1:1 ratio of thiobarbituric acid (TBA) 0.37%, trichloroacetic acid (TCA) 15%, and hydrochloric acid (HCl) 0.25 N reagent. All the tubes were placed in a boiling water bath for 30 minutes and cooled. The amount of malondialdehyde formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535 nm against the reference blank. Percentage inhibition was calculated using the equation.[34]

% lipids peroxidation inhibition = $(A_o - A_1)/A_o \times 100$

Where, A_o is the absorbance of the control and A_1 is the absorbance of the sample.

Estimation of Superoxide Dismutase (SOD)

The SOD activity was determined by the method of Patil *et al*. In this method, the brain homogenate was assayed by monitoring its ability to scavenge superoxide

radicals generated by auto-oxidation of pyrogallol in the alkaline medium. Each 3 mL reaction mixture contained 2.8 mL of potassium phosphate buffer (0.1 M, pH 7.4), 0.1 mL tissue homogenate, and 0.1 mL pyrogallol solution (2.6 in 10 mm HCl). The rate of increase in the absorbance at 325 nm was recorded for 5 minutes with a 30 seconds interval. One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation per 3 mL of the assay mixture.[35]

Estimation of Total Protein Concentration

Protein content in the brain homogenate was measured using the biuret method. In this method, 3 mL of biuret reagent was added in each sample test tube, then add 0.3 mL of brain homogenate, kept at 37°C for 10 minutes, and absorbance measured at 540 nm.

Statistical Analysis

All the values of the experimental results are expressed as mean ± Standard Error of Mean (SEM), and statistical significance between the groups was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's post-test ($p < 0.01$ was considered statistically significant). Statistical analysis was carried out using GraphPad InStat 5.0 (GraphPad Software).

RESULTS

The study analyzed the effect of 7-HF on behavioral and biochemical significance in Parkinson's disease in Swiss albino mice, as shown in Tables 1 to 3. 7-HF, known for various pharmacological activities, like anti-nociceptive, anti-inflammatory, anti-diabetic, anti-trypanosomal, anti-leishmanial, etc., in the reported literature. In a behavioral study, the effect of 7-HF on the rotarod test showed significant performance in the rotarod test in a dose-dependent manner, as compared with the vehicle-treated group. The highest dose (100 mg/kg, $p < 0.001$) treated animals, spent maximum time on the rotarod equipment, while the lowest dose-

Table 1: Data showing effect of 7-HF on open field test in reserpine-induced Parkinson

Treatment	*Dose (mg/kg body weight)*	*Mean number of line crossing in open field test*	*Mean number of rearing in open field test*	*Mean number of grooming in open field test*	*Mean of immobility time (sec)*
Negative control	-	53.33 ± 1.667***	53.33 ± 1.667***	55 ± 1.826***	45 ± 3.651***
Vehicle treated	-	6 ± 0.7303#	6 ± 0.7303#	8 ± 0.3651#	160 ± 20#
Standard	275	33 ± 0.9661***	33 ± 0.9661***	16 ± 0.7303***	42 ± 2.486***
7-hydroxyflavone	25	12 ± 0.8761*	12 ± 0.8211**	49.83 ± 1.922**	96 ± 4.072***
7-hydroxyflavone	50	25 ± 1.826***	25 ± 1.826***	30 ± 1.826***	60 ± 0.7303***
7-hydroxyflavone	100	36 ± 2.386***	35.83 ± 2.386***	47 ± 1.317***	50 ± 0.681***

Data values are expressed as mean ± SEM; ***$p < 0.001$; **$p < 0.01$; #vehicle-treated

Table 2: Data showing effect of 7-HF on different behavioral responses in reserpine-induced Parkinson

Treatment	*Dose (mg/kg body weight)*	*Mean fall off time (sec) in rotarod test*	*Mean time spent on bar (sec) in catalepsy test*	*Mean number of counts/5 minutes in total locomotor activity*
Negative control	-	5.33 ± 2.472***	2 ± 0.3651***	230 ± 8.563***
Vehicle treated	-	4 ± 0.3651#	57.5 ± 3.819#	12 ± 0.7303#
Standard	275	45.83 ± 1.537***	10 ± 0.7303***	74 ± 1.528***
7-hydroxyflavone	25	12 ± 0.7303**	35 ± 1.826***	27.5 ± 2.141*
7-hydroxyflavone	50	24.17 ± 1.721***	21.33 ± 0.988***	50 ± 1.826***
7-hydroxyflavone	100	46.67 ± 1.626***	10 ± 0.7303**	73.67 ± 1.308***

Data values are expressed as mean ± SEM; ***$p < 0.001$; **$p < 0.01$; #vehicle-treated

Table 3: Data showing effect of 7-HF on chimney test in reserpine-induced Parkinson

Treatment	*Dose (mg/kg body weight)*	*Mean time of backward movement out of cylinder (sec) in chimney test*
Negative control	-	8 ± 0.3651***
Vehicle treated	-	142 ± 0.9189#
Standard	275	21.6 ± 4.047***
7-hydroxyflavone	25	10 ± 2.056***
7-hydroxyflavone	50	1.8 ± 1.826***
7-hydroxyflavone	100	3.6 ± 3.651***

Data values are expressed as mean backward movement out of cylinder ± SEM; ***$p < 0.001$; **$p < 0.01$; #vehicle-treated

treated (25 mg/kg, $p < 0.01$) minimum. In the open field test, the highest dose (100 mg/kg, $p < 0.001$) treated animals showed a maximum number of line crossing, rearing, and grooming, while the lowest dose-treated (25 mg/kg, $p < 0.005$) showed minimum. The duration of immobility was minimum at the highest dose (100 mg/kg, $p < 0.001$) treated animals, while 25 mg/kg, $p < 0.001$ treated showed maximum.

In catalepsy reduction in cataleptic score in a dose-dependent manner, the highest dose (100 mg/kg, $p < 0.01$) treated animals showed minimum cataleptic scores in bar test, while the lowest dose (25 mg/kg, $p < 0.001$) treated showed maximum. In the total locomotor activity, the highest dose (100 mg/kg, $p < 0.001$) treated animals showed the maximum number of counts in actophotometer, and the lowest dose (25 mg/kg, $p < 0.05$) treated showed minimum. In the chimney test, the highest dose (100 mg/kg, $p < 0.001$) treated animals takes minimum time to move backward out of the cylinder, while the lowest dose (25 mg/kg, $p < 0.001$) takes maximum time. Standard drug syndopa (275 mg/kg) showed similar and significant results on Reserpine treated animals. Reserpine causes neurodegenration animals because they may be a reversal of catatonia. It can be said that the test compound may have the same mode of action as the standard drug. The effect of 7-HF on the biochemical test is shown in Tables 4 and 5. In catalase activity, the highest dose (100 mg/kg, $p < 0.001$) treated animals showed maximum inhibition in the production of free radical, and the lowest dose (25 mg/kg, $p < 0.001$) showed a minimum. It means that the test compound significantly increases the catalase activity as in the increment of its dose. In the GSH test, the 7-HF showed significant inhibition percentage production of free radical in a dose-dependent manner. The highest dose (100 mg/kg, $p < 0.001$) treated animals showed maximum inhibition of free radical in the GSH activity, and the lowest dose (25 mg/kg, $p < 0.001$) showed a minimum. In LPO activity, the percent inhibition of the lipid peroxidation in the brain tissue of 7-HF treated mice was obtained in a dose-dependent manner. The highest dose (100 mg/kg, $p < 0.001$) treated animals showed maximum lipid peroxidation (LPO) inhibition, and the lowest dose (25 mg/kg $p < 0.001$) showed a minimum. In SOD activity, the highest dose of 7-HF (100 mg/kg, $p < 0.001$) treated animals showed maximum inhibition in the production of free radical, and the lowest dose (25 mg/kg $p < 0.001$) showed a minimum. Standard drug (syndopa, 275 mg/kg) treated animals showed the same effects as 7-HF. Thus, test and standard drugs may be the reversal of oxidative stress or prevent the generation of reactive oxygen species (ROS). All animals treated with 7-HF showed a significant increase in total protein concentration in a dose-dependent manner, as compared to the vehicle-treated group. Standard drug (syndopa, 275 mg/kg) treated animals also showed maximum protein concentration.

DISCUSSION

Shape-based and e-pharmacophore based virtual screening protocols were used to retrieve the potential hits from the molecular databases (ASINEX and Sigma Compound Library). For shape-based screening, some antiparkinson's agents belonging to different

Table 4: Data showing effect of 7-HF on *in vitro* test parameter in reserpine-induced Parkinson

Treatment	*Dose (mg/kg body weight)*	*Mean percent inhibition in production of free radical in SOD*	*Mean percent inhibition in production of free radical in GSH*	*Mean percent inhibition in LPO*
Negative control	-	$0 \pm 0^{\#}$	$0 \pm 0^{\#}$	$0 \pm 0^{\#}$
Vehicle treated	-	21.42 ± 2.609	14.68 ± 0.6486	23 ± 0.8563
Standard	275	$78.38 \pm 2.803^{***}$	$74.82 \pm 0.3368^{***}$	$74 \pm 0.7303^{***}$
7-hydroxyflavone	25	$51.41 \pm 2.248^{***}$	$31.76 \pm 1.103^{***}$	$39.67 \pm 2.028^{***}$
7-hydroxyflavone	50	$65.81 \pm 0.8862^{***}$	$46.38 \pm 0.6025^{***}$	$52 \pm 1.461^{***}$
7-hydroxyflavone	100	$72.45 \pm 0.7523^{***}$	$71.17 \pm 0.7841^{***}$	$64 \pm 0.73.03^{***}$

Data values are expressed as mean ± SEM; $^{***}p < 0.001$; $^{**}p < 0.01$; $^{\#}$negative control

Table 5: Data showing effect of 7-HF on *in vitro* test parameter in reserpine-induced Parkinson

Treatment	*Dose (mg/kg body weight)*	*Mean percent inhibition in production of free radical in CAT*	*Mean concentration in µg/mL in total protein concentration*
Negative control	-	$84 \pm 1.095^{***}$	1,750 ± 54.77
Vehicle treated	-	$24 \pm 0.7303^{\#}$	$1,600 \pm 12.43^{\#}$
Standard	275	$77.67 \pm 0.9545^{***}$	$201.7 \pm 36.51^{***}$
7-hydroxyflavone	25	$50 \pm 1.826^{***}$	$806.7 \pm 32.11^{***}$
7-hydroxyflavone	50	$65 \pm 1.824^{***}$	$1,233 \pm 55.78^{***}$
7-hydroxyflavone	100	$72 \pm 0.7307^{***}$	$2,080 \pm 62.93^{***}$

Data values are expressed as mean ± SEM; $^{***}p < 0.001$; $^{**}p < 0.01$; $^{\#}$vehicle-treated

pharmacological categories and with a good range of biological activity were selected from literature as shape query or reference molecules (apigenin, luteolin, pramipexole, rasagiline, levodopa, and tolcapone). However, the crystal structure of some molecular targets, such as, monoamine oxidase, acetylcholinesterase, and catechol-O-methyl transferase in complex with their respective inhibitors, were taken from PDB, to develop e-pharmacophore hypotheses. Furthermore, both the approaches were collectively used to retrieve the hits from the ASINEX database and submitted for molecular docking to investigate their binding affinity with the antiparkinson's target. The resulting hits were ranked based on shape similarity, fitness score, XP g-score, and conserved interactions. Moreover, *in silico* toxicity assessment of the retrieved hits was performed using Osiris and Lazar programs. Finally, the most potential candidate, 1-[4-(4-fluoro-benzyloxy-phenyl]-pyrolidine-3-carboxyllic acid amide, obtained from the ASINEX compound database was selected for biological evaluations, based on its *in silico* performance.

Since top-scoring hits obtained from the ASINEX database were found to be very costly, therefore, on a second priority basis some more hits were screened from Sigma Compound Library, which is commercially available at a reasonable cost. Shape-based screening of Sigma Aldrich Compound Library against selected query ***mol***ecules was performed and the obtained hits were filtered based on shape similarity, XP g-score, conserved interactions, and toxicity parameters. Finally, 7-hydroxy 2 phenyl-4H-chromen-4-one, i.e., 7-HF was selected for *in vivo* and *in vitro* studies.

In the present study, 7-HF was evaluated for its antiparkinson activity by reserpine-induced Parkinson's model of mice. Reserpine act by depleting central catecholamine stores in dopaminergic neurons and produce oxidative stress by the generation of free radicals.[36] A molecular structure of the 7-HF has an extensive electron conjugated pie system that interacts with various reactive oxygen intermediates, such as, superoxide anion, hydroxyl, and peroxyl radicals, (generated by the reserpine) efficiently quenches singlet oxygen.[37] The treatment of 7-HF may have protected the dopaminergic cells from degeneration in the SN of mice brain, consequence enhanced motor co-ordination, locomotion, decreased cataleptic, and chimney test scores. 7-HF significantly improved the behavioral activities, as compared to vehicle and standard treated groups. SOD and catalase (CAT) are the major enzymes that scavenge the reactive oxygen species in the biological system.[38] Here, a concomitant rise in both SOD and CAT in the brain homogenate of the 7-HF treated animals, indicates excellent protection from oxidative stress. GSH level is also depleted during oxidative stress, making the cells prone to oxidative damage,[39] which was also found to be enhanced by the treatment of 7-HF. In terms of percent inhibition, the lipid peroxidation reaction was inhibited in a dose-dependent manner in 7-HF treated groups. The concentration of total protein in the brain was also found to be elevated in 7-HF treated animals in a dose-dependent manner. In the present study, syndopa was used as a standard drug. It is a combination of carbidopa and levodopa, acts by inhibiting dopa decarboxylase enzyme. It is prescribed for the treatment of Parkinson's disease.[40] Interestingly, in behavioral and biochemical studies, 7-HF showed approximately equivalent effects as compared to syndopa.

In summary, findings indicate the attempt to evaluate the antiparkinson effect of 7-HF in reserpine-induced Parkinson's model of mice was successful. The behavioral and biochemical results obtained from this study disclosed a definite neuroprotective role. It is also clear that 7-HF showed potent and effective antiparkinson activity in a similar way, as standard.

CONCLUSION

In summary finding indicate the attempt to evaluate antiparkinson effect of 7-hydroxyflavone in the reserpine induced Parkinson model of mice was successful. The behavioral and biochemical results obtained from this study disclosed a definite neuroprotective role. It is also clear that 7-hydroxyflavone showed potent and effective antiparkinson activity in the similar way as standard.

FUTURE PROSPECTIVE

In the perspective of Parkinson's disease, drugs that serve symptomatic relief, as well as, delay the progression of the disease could be a rational approach to treat Parkinson's-like neurodegenerative disorders. However, a detail pharmacokinetic profile along with the safety of 7-HF needs to be investigated in further studies. There is also a need for *in vivo* toxicity estimation in order to justify *in silico* toxicity prediction.

ABBREVIATIONS

PD-Parkinson's disease; SN-substantia nigra; ACh-acetylcholine; DDC-dopadecarboxylase; MAO B-monoamine oxidase; CAT-catalase; GSH-reduced glutathione; SOD-superoxide dismutase, 7HF-7-hydroxyflavone; COMT-catechol-O-methyl transferase; AChE-acetylcholinesterase; XP-extra precision; HTS-high throughtput screening; HTVS-high throughtput virtual screening; VSW-virtual screening workflow; RMSD-

root mean square deviation; PDB-Protein Data Bank; *DJ-1*-protein deglycase; *PTN*-protein tyrosine phosphate; *LRRK2*-leucine rich repeat kinase 2.

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