




Neurobehavioral and neuroprotective role of captopril in the rotenone model of rat Parkinsonism

Prakash KG^{*1}, Bannur BM², Madhavrao C³, Saniya K¹, Sudha M J³, Viveka S¹

¹Department of Anatomy, Azeezia Institute of Medical Sciences and Research, Kollam, Kerala, India

²Department of Anatomy, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state, BLDE University, Bijapur, Karnataka, India

³Department of Pharmacology, Azeezia Institute of Medical Sciences and Research, Kollam, Kerala, India

Article History:	ABSTRACT
Received on: 15.07.2019 Revised on: 20.10.2019 Accepted on: 26.10.2019 <i>Keywords:</i>	 <p>Angiotensin-converting enzymes are increasingly being tested in therapeutics of Parkinsonism. The objective of the present study was to evaluate the behavioral changes and neuroprotective role of captopril in the rotenone model of Parkinsonism in rats. Adult Wistar albino rats were divided into four groups of six each. Parkinsonism was induced with rotenone (3 mg/Kg intraperitoneal) in three groups. The experimental group was treated with captopril (20 mg/kg intraperitoneal). The effects were compared with a standard group treated with levodopa (12 mg/Kg) and Benserazide (3 mg/Kg). Behavioral effects were evaluated by the rotarod test, spontaneous locomotor activity, hole board test, forced swim test, and tail suspension test. Neuroprotection was noted with an estimation of glutathione and lipid peroxidation from rat brain homogenate. Levels of dopamine, serotonin, and GABA were also noted. Haematoxylin and eosin-stained sections of the brain evaluated for any histoarchitectural changes. Rats pre-treated with captopril have shown a significant increase in the duration of stay in the rotarod test, a significant increase in the number of head dipping in hole board test, significant lower duration of immobility in forced swim test and tail suspension test. Captopril has shown significant improvement in motor coordination (as evidenced through rotarod test), exploratory behavior (hole board test), depression (forced swimming test, and tail suspension test). Captopril significantly reduces oxidative stress conditions. Captopril has not shown major histoanatomical changes in the rotenone model. Angiotensin-converting enzyme inhibitors; neuroprotection; dopaminergic neurons; Parkinsonism; rotenone model</p>
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*Corresponding Author

Name: Prakash KG
 Phone: +9196452 16564
 Email: drprakashkg@gmail.com

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INTRODUCTION

With a deeper understanding of the pathogenesis of Parkinsonism, the newer avenues of therapeutics concentrating on the prevention of neuronal loss are being explored. There is a constant addition of evidence to establish a stronger association of brain RAS system with neurodegenerative disorders. (Wright and Harding, 2012) Angiotensin I, II, III, and IV are shown to influence the vasoconstriction, neuroinflammation, oxidative stress, and apoptosis. They bring these effects through angiotensin 1-7 and angiotensin 3-7 subsidiaries

acting via AT1 receptors. Conversely, the same angiotensin derivatives acting via AT2 and AT4 receptors are shown to produce angiogenesis and bring about anti-inflammatory, anti-oxidative, and anti-apoptotic effects (Yamamoto *et al.*, 2010). A number of studies report evidence of a link between brain RAS and PD. A nationwide cohort study evaluating the use of anti-hypertensive in more than 65 thousand patients with PD in Taiwan has shown that the use of ACE and ARBs is associated with a decreased incidence of PD (Lee *et al.*, 2014). Recent studies have also shown involvement of brain RAS with other neuronal conditions like stress and anxiety (Peng *et al.*, 2002), depression, cognitive dysfunction (Saab *et al.*, 2007), and alcohol intake (Maul *et al.*, 2005). AT1 receptor inhibition has been shown to be associated with improved learning, spatial memory, and motor coordination (Hellner *et al.*, 2005; Kerr *et al.*, 2005). Patients with PD concurrently receiving ACE inhibitors perindopril show improvement in motor symptoms. (Reardon *et al.*, 2000) Louise *et al.*, after analyzing 60 PD patients who are taking ACEI for hypertension, concluded that ACEI might be independently associated with a reduced probability and a reduced number of falls among patients with PD.

Many studies have established the beneficial effects of perindopril in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of Parkinsonism. Sonsalla *et al.* have shown captopril protects the striatum from MPTP, and chronic administration of captopril protects the dopaminergic neurons from degeneration in rats. (Sonsalla *et al.*, 2013) Muñoz *et al.* have shown that captopril, in addition to influence the motor co-ordination of MPTP induced Parkinsonism, brings about the reduction in oxidative stress. They claim that this influence on oxidative stress is by inhibition of angiotensin activated NADPH-dependent oxidases. (Muñoz *et al.*, 2006) Katrina *et al.* have shown that a four-week treatment with perindopril improves the clinical features of PD with a reduction in 'on phase' dyskinesia. (Reardon *et al.*, 2000) Neuroprotective effects of perindopril have also been documented in the rat MPTP model. (Kurosaki *et al.*, 2004) There is a report on a combination of aspirin and nimodipine effective in neuroprotection and in improving motor symptoms in the MPTP model among rats. (Ambhore *et al.*, 2014) Overall, in conclusion, a Cochrane review article finally opines that there is a lack of evidence for the use of antihypertensive drugs for either primary or secondary prevention of PD. (Rees *et al.*, 2011) Authors of this review call for more studies to establish the role of ACEI and ARBs in therapy and prevention of PD.

With this background of the angiotensin system variably shown to effect the Parkinsonism disease process and clinical manifestations, the beneficial effects of captopril were evaluated in the present study. The objectives of the present study were to evaluate the neurobehavioral effects of captopril in the rotenone model and to evaluate the neuroprotective role of captopril in terms of improvement in enzymatic oxidative stress markers and histological changes in various parts of the rat brain.

MATERIALS AND METHODS

Induction of Parkinsonism and grouping of animals

Healthy adult Wistar albino rats of either sex weighing 180-250g were selected. All animals were obtained from animal house, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state & KMCH College of Pharmacy, Coimbatore, Tamil Nadu. All the animals were kept at room temperature. Rodents were allowed to acclimatize in standard conditions of 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. Institutional animal ethics committee, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state, (with CPC-SEA, India registered) (approval letter number: 33/16, dated-16.01.2016) and also Institutional animal ethics committee, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, (approval letter number: KMCRET/PhD/21/16-17, dated-22.02.2016) approved the study before the start of the study.

The Rotenone (Sigma Chemicals, Mumbai) solution was freshly prepared at 3 mg/kg. The Rotenone was dissolved in dimethylsulfoxide and adjusted to pH 7.4 with potassium hydroxide. Rotenone injected i.p at the dose of 3 mg/kg body weight, 7 days. The solution was used immediately after preparation, as it was stable only for a period of 24 hours at 25°C Table 1.

Animals that died after the intraperitoneal injection were excluded from the analysis. Animals that failed to experience the parkinsonian symptoms were also excluded. Animals that died within minutes of injection and that did not experience parkinsonian symptoms were excluded from the analysis. All animals that did not survive 7 days of intraperitoneal injections were excluded from the study.

Behavioral analysis

Motor functions were evaluated by rotarod test, spontaneous locomotor activity. Exploratory behavior was evaluated by the hole board test. Depres-

Table 1: Four groups (each having 6) of rats that were used in the study (n=24)

Groups	Group specifications
Group 1	Control group - Equivalent normal saline i.p
Group 2	Negative control: Rotenone (3 mg/Kg BW i.p)
Group 3	Positive control: Levodopa (12 mg/Kg) and Benserazide (3 mg/Kg BW i.p) + Rotenone (3 mg/Kg BW i.p)
Group 4	Captopril (20mg/kg BW i.p) + Rotenone (3 mg/Kg BW i.p)

sion influences on behavior were studied with the forced swim test and tail suspension test. Elevated plus maze test was used for analyzing anxiety influences on behavior.

Rotarod test

A motor co-ordination test was conducted by placing the rats on the horizontally placed rotating rod. Rats were trained for three minutes in a trial at 25 rpm for a week before the administration of the drug. After each trial, a 5 min rest period was given to alleviate stress and fatigue. Motor coordination was tested by comparing the latency to fall on the very first test between treatment groups. The time taken by animals to fall from the rotating rod was noted. The length of time (duration) the animal stays on the rod without falling gives a measure of their coordination, balance, physical condition, and motor-planning. A cut off time of 240s (4 minutes) was fixed, and each animal performed 3 separate trials at 10 min interval. (Deacon, 2013)

Spontaneous locomotor activity

The spontaneous horizontal activity was measured using an actophotometer that operates on photoelectric cells connected with a counter. The apparatus was placed in a sound-attenuated and ventilated room during the testing period. All the rats were placed individually in the activity cage for 3 min to habituate them before starting the actual locomotor activity task for the next 3 min. The basal activity score was noted. The units of the activity counts were arbitrary and based on the beam breaks by the movement of the animal. Counts per ten min are used as an index of locomotor activity. (Sgroi et al., 2014)

Hole board test

Rats were placed on a 25 cm elevated wooden board (40 cm X 40 cm) with 16 holes. Each hole was 3 cm in diameter, spaced at regular intervals. Rats were placed on the corner of the apparatus and were observed for the next 5 min for the number of head dipping. A head dipping is counted when the animal introduces its head into any hole of the box up to the level of the ears. The apparatus

was thoroughly cleaned between each subject. The decrease in anxiety shows increased exploration of the holes. (Brown and Nemes, 2008)

Elevated plus maze test

The plus-maze for mice consisted of two perpendicular open arms (30×5cm) and two closed arms (30×5×15cm) also in a perpendicular position. The rats were placed at the junction of the maze with four arms, facing an open arm. The duration in each arm was recorded by a video — tracking system and by an observer simultaneously for 5 minutes. The duration of the test period was 10 minutes, the number of entries into the open arm, number of entries into the closed arm, the time spent in open arm, and closed arm were noted.

Forced swim test

Rats were placed in an open cylinder having a diameter of 10 cm and a height of 25 cm with water up to 15 cm at 25 ± 1°C. The rats were forced to swim in this small space. This induces a circumstance of immobility behavior. When the animal ceases to struggle to get out and attains a state of floating with immobility, with minimal movements to keep the head above water, the time was measured. The total duration of the FST was fixed at 240 seconds (4 minutes). The entire duration for each animal was video recorded and analyzed later in a PC. During behavioral analysis, the time each mice spends mobile is measured and noted. The total mobility time is subtracted from 240 seconds. This represents the immobility time. This method was adopted as it is better to note the movements than the lack of movements. Any movement other than those necessary to balance to the body and keep the head above the water was considered as mobility or movement. (Can et al., 2012a)

Tail suspension test

Rats were gently lifted by their tails and slowly placed on a support. The grid was inverted, resulting in rats to hang from the grid upside down. Care was taken to prevent the rat from falling down and getting injured by mounting the grid at 20 cm from the ground level. The grid was also provided with

a 3-inch wall to prevent rats from traversing to the higher levels of the grid. The animals were made to stay on the grid for 240 seconds (4 minutes). (Can et al., 2012b) The tail hanging time was estimated in 10 chances with a 1-minute interval between the trials. The immobility time was defined as the time during which the animal was hanging passively. This was a measure of depression.

Dissection of brain and processing of the two hemispheres

All the rats were anesthetized using thiopental sodium (50 mg/kg) after 24 hours of the behavioral monitoring. All rats were sacrificed by cervical decapitation. The brain was dissected out of the cranial cavity. Each brain was Hemi sectioned along the longitudinal fissure into right and left halves. One hemisection was homogenized and used for the estimation of neurotransmitters and oxidative stress markers. Another hemisection was fixed with formalin and used for histological study.

Enzymatic antioxidant activity

Estimation of reduced glutathione

To 250 μL of tissue homogenate taken in 2 ml Eppendorf tube, 1 mL of 5% TCA was added, and the above solution was centrifuged at 3000 g for 10 min at room temperature. To 250 μL of the above supernatant, 1.5 ml of 0.2 M phosphate buffer was added and mixed well. 250 μL of 0.6 mM of Ellman's reagent (DTNB solution) was added to the above mixture, and the absorbance was measured at 412 nm within 10 min. A standard graph was plotted using glutathione reduced solution (1 mg/mL), and GSH content present in the tissue homogenates was calculated by interpolation. The amount of glutathione expressed as $\mu\text{g}/\text{mg}$ protein. (Tipple and Rogers, 2012)

Lipid peroxidation assay

To 100 μL of the tissue homogenate, 2 mL of (1:1:1 ratio) thiobarbituric acid reagent (thiobarbituric acid 0.37%, 0.25 N hydrochloric acid, and 15% trichloroacetic acid) was added and mixed. The above content was incubated in a boiling water bath for 15 min, cooled and centrifuged at 3500 rpm for 10 min at room temperature. The pink color developed was estimated at 535 nm against a reagent blank, in a spectrophotometer. LPO was expressed as nmol of MDA/mg protein. (Devasagayam et al., 2003)

Myeloperoxidase (MPO) activity estimation

MPO was measured with the tetramethylbenzidine method. To 80 μl 0.75 mM H_2O_2 and 110 μl TMB solution, a 10 μl sample were added. The mixture was incubated at 37°C for 5 minutes. 50 μl 2

M H_2SO_4 stopped the reaction, and absorption was measured at 450 nm to measure MPO activity. (Pulli et al., 2013)

Catalase activity

The homogenate mixture was mixed with 1.95 mL of 50mM phosphate buffer, and 1 mL of 30mM hydrogen peroxide was added. The catalase activity was measured as 240nm at 15 seconds intervals. The catalase activity was estimated according to change in absorbance/minute of catalase with respect to the extinction coefficient of hydrogen peroxide (0.071 mmol cm^{-1}). Catalase activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein. (Bhangale and Acharya, 2016)

Superoxide Dismutase (SOD) Level

0.1 mL of the supernatant of homogenate mixture was mixed with 0.1 mL Ethylenediaminetetraacetic acid, EDTA (1×10^{-4} M), 0.5 mL of carbonate buffer and 1 mL of epinephrine (1 mM). The spectrophotometric measurement of the mixture was measured at 480nm for 3 minutes. The SOD activity was expressed in terms of U/min/mg. (Bhangale and Acharya, 2016)

MAO — A and MAO — B estimation

The brain homogenate mixtures were added with 4 mM 5-HT and 2 mM b-PEA as specific substrates for MAO-A and MAO-B respectively. The final volume was made as 1 ml by adding 100 mM sodium phosphate buffer. The tubes were incubated at 37°C for 20 minutes and stopped by adding 1 M HCl (200 ml). The products were extracted with 5 ml of butyl acetate (for MAO-A) and cyclohexane (for MAO-B), respectively. This extract was measured at a wavelength of 280 nm for MAO-A assay and 242 nm for MAO-B assay with a spectrophotometer, respectively. (Yu et al., 2002)

Estimation of total protein

To 0.1 ml of the homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added, and allowed to stand at the room temperature for 10 minutes. To this, 0.5 ml of Folin's reagent was added. After 20 minutes, the color developed was measured at 640 nm. The level of protein present was expressed as mg/g/tissue. (O'callaghan et al., 1999)

Histopathological evaluation

Hemisections of the brain were fixed with 10% formalin for 48 hours. Paraffin blocks were prepared. 5- μm -thick sections were prepared and processed for histopathological and immunohistochemical studies.

H and E stained slides

Showing regional changes in the frontal lobe, temporal lobe, basal ganglia hippocampus, and cerebellum were evaluated for histoarchitectural changes.

Bcl-2 immunohistochemistry

Immunohistochemistry was done with 5- μ m-thick pre-treated sections that were placed on L-lysine slides. For anti-apoptotic oncoprotein, Bcl-2 retrieval, the slides were immersed in sodium citrate 0.1M. Slides were preheated in a 750 W microwave oven for 7 min. The mouse monoclonal antibody to Bcl-2 (Bio SB, BioSciences For the World, CA 93117, USA) diluted in 1:100 phosphate buffer saline was used in the study. The slides covered with antibody were placed in a solution jar-containing buffer. Slides were covered with peroxidase blocking and incubated for 10 minutes. Washed with deionized water and buffer periodically. This was followed by washing with buffer solution 3 times. Then slides were placed in substrate 3,3'-Diaminobenzidine (DAB) solution for ten minutes and later washed with buffer. Hematoxylin counterstained the slides. (Malusecka *et al.*, 2006). All slides were evaluated for Bcl-2 immunohistochemistry, and pathology expert opinion was obtained. The scoring system used in the present study is adopted from criteria defined by Tsuyama *et al.* for Bcl-2 quantification in blood dyscrasias, especially B-cell related leukaemia. (Tsuyama *et al.*, 2017)

Statistical analysis

Data obtained from each model were tabulated separately and subjected to statistical analysis. Data were tabulated and presented as tables and diagrams. For all continuous parameters, mean \pm standard deviation was calculated for each group. A comparison of the data was done by one way ANOVA test. All the parameters were compared with control group values. All calculations were done with the software SPSS V 20 32bit. A p-value of less than 0.05 was taken as significant.

RESULTS AND DISCUSSION

Four percent of the rats that were initially recruited died during the induction of Parkinsonism, and five percent of the rats died during the subsequent evaluation of the properties of Angiotensin receptor blockers. Seven percent of the rats were excluded from the study because of insufficient induction of Parkinsonism in various models.

Neurobehavioral studies

The mean time stays on rotarod in rotenone-induced Parkinsonism rats and in rats pre-treated

with levodopa were significantly decreased in comparison to the control animals. Group pre-treated with captopril had significantly increased the duration of stay on the accelerating rotarod in comparison with the control group Table 2. The number of counts per 10 minutes in rats pre-treated with captopril and levodopa were significantly reduced in spontaneous locomotor activity. Exploratory behavior assessment with the hole board test showed a significant increase in the number of head poking characteristics in rats rotenone and the captopril group in comparison to the control group Table 2. This shows that rats pre-treated with these drugs had improved exploratory behavior and were less anxious. Rotenone induced. Parkinsonism rats spent more time in the closed arm of the plus-maze than open arms as compared to the control rats. Rats pre-treated with captopril revealed significant improvement in anxiety-like behavior. No statistically significant difference in the time spent on the plus maze (both in the open and closed arm) between the captopril pre-treated group and rotenone groups on day 7. Both groups had significantly increased the time spent in open and closed arms in comparison to the control group. Rotenone induced rats had significant higher immobility time, indicating the depressive behavior after exposure to rotenone. The rats pre-treated with levodopa had more immobility time compared to the control group. The test group rats exposed to captopril had significantly less immobility time in comparison to the rotenone group, indicating anti-depressant effects of the test drug Table 2. Rotenone induced rats had higher immobility time compared to the control group. The rats pre-treated with the levodopa, and captopril had lower immobility time in comparison to the rotenone group. However, the time was not reduced below the control group.

Neurotransmitters estimation

Dopamine levels were significantly higher in the rats pre-treated with levodopa and captopril. There was no significant difference between dopamine levels in the brain hemisections of rotenone-induced Parkinsonism rats and control rats. Serotonin levels in the rotenone-induced rats were significantly less than the control group Table 3. Both levodopa and captopril groups had significantly higher levels of serotonin in comparison to the control group. GABA levels in the captopril pre-treated rats were significantly lower than the control group. Both levodopa and captopril groups had significantly lower levels of acetylcholine than the control group rats. Glutamate levels in both levodopa and captopril groups were significantly lower than the control group. Levodopa group had the lowest overall levels of the glu-

Table 2: Tabulation of anxiety and depression behavior among six groups (n=6) of rats of the rotenone model

Group	Control group	Rotenone group	Rotenone + levodopa group	Rotenone + Captopril
Rotarod test (in seconds)	186.8 ± 4.5	123.6 ± 8.2†	159.3 ± 2.2†	194 ± 6.6*
Actophotometer (number of counts /10 min)	450 ± 8.1	384 ± 9†	410.3 ± 14.6*	402.8 ± 9.5*
Hole board test (number of times head dipping in the hole / 4 min)	14.6 ± 0.9	10.5 ± 0.7†	23.8 ± 1.1†	20 ± 0.7†
Elevated plus maze (Number of entries in the open arm)	114.3 ± 3.6	80.1 ± 3.6	149.8 ± 7.5†	113.1 ± 3.5
Elevated plus maze (Time spent in the open arm)	178.5 ± 11.4	124.3 ± 3.3*	153.6 ± 3.9†	144.3 ± 4.4*
Elevated plus maze (time spent in the closed arm)	139.8 ± 5.7	185.5 ± 6.5†	150.5 ± 3.3†	152.9 ± 3.9†
Forced swim test (Immobility time in sec)	131.6 ± 1.5	192.5 ± 4.6†	156.3 ± 2.1	142.3 ± 4.8†
Tail suspension test (Immobility time in sec)	120.8 ± 3.6	204.1 ± 2.1†	142.6 ± 2.6	154.5 ± 4.8†

Expressed as mean ± standard deviation, †p<0.001, *p<0.05 as compared to control group

Table 3: Tabulation of neurotransmitters levels in rotenone model, each group (n=6)

	Control group	Rotenone group	Rotenone + levodopa group	Rotenone + Captopril group
Dopamine	14.55 ± 0.56	13.17±1.7	41.45±2.8†	22.21± 5.2†
Serotonin	221.4 ± 15.7	110.4±3.6†	349.1±60.7†	231.5±27.4*
GABA	3.17 ± 0.5	4.5±1.1†	3.5±2.2	3.0±0.8*
ACH	127.3 ± 3.5	52.2±1.4†	32.1 ± 3.3†	30.5 ± 5.2†
Glutamate	142.7 ± 4.8	140.6±4.8	63.3±4.4†	136.6±3.3*
Total protein	23.8 ± 1.9	23.2 ± 2.5	32.1 ± 2.1†	25.1 ± 1.4

Dopamine (pg/g tissue), serotonin (pg/g tissue) Gamma Amino Butyric Acid (GABA, ng/gtissue) acetylcholine (ACH, mol/min/μg tissue) glutamate (μg of monoamine/gtissue) and total protein (mg/100g) of rat brain tissue from each group (n=6) expressed as mean and standard deviation, †p<0.001, *p<0.05 as compared to control group

tamate.

Oxidative stress indicators

Glutathione levels were significantly lower than the control group in all the groups. Lipid peroxidase levels were significantly increased in both levodopa and captopril groups in comparison to the control group Table 4. Myeloperoxidase levels were significantly increased in both levodopa and captopril groups in comparison to the control group. Catalase levels were significantly increased in both levodopa and captopril groups in comparison to the control group. SOD levels were increased significantly in both levodopa and captopril groups in comparison to the control group. MAO – A levels were significantly decreased in the rotenone and captopril group in comparison to the control group. MAO – B levels were significantly increased in the captopril group in comparison to the control group Table 4

Histological studies

Sections of rat brain with rotenone-induced Parkinsonism showed hyperchromatic pyknotic nucleated neurons in the cerebrum with edema Figure 1. There were no remarkable changes in the substantia nigra and corpus striatum. Immature and granular cells were noted in the cerebellum. Rat brain treated with levodopa and benserazide showed vacuolations in the cerebrum, striatal edema. The cells in the thalamus were hyperchromatic and pyknotic Figure 2. The cells having surrounding vacuoles were lightly stained and small. Rat brain treated with captopril showed normal cerebellum and thalamus histology. Cells in the cerebrum had vacuolations Figure 3. Corpus striatum was edematous.

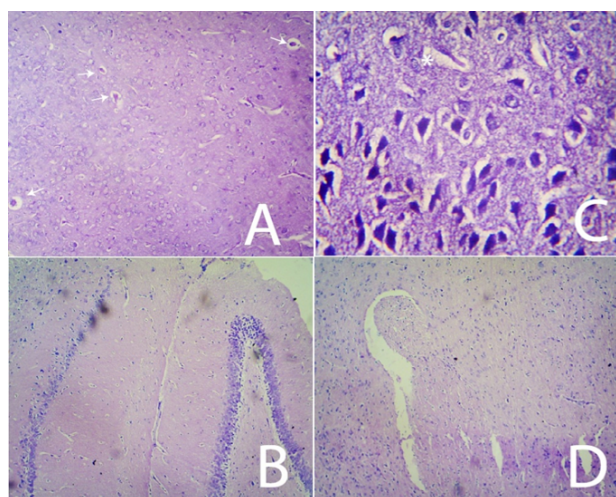


Figure 1: Photomicrograph of rotenone-induced Parkinsonism rat brain showing normal cerebellum (B), normal thalamus (B), pyknotic nuclei (white arrow) in the cerebrum and edema (*) in the corpus striatum (C)

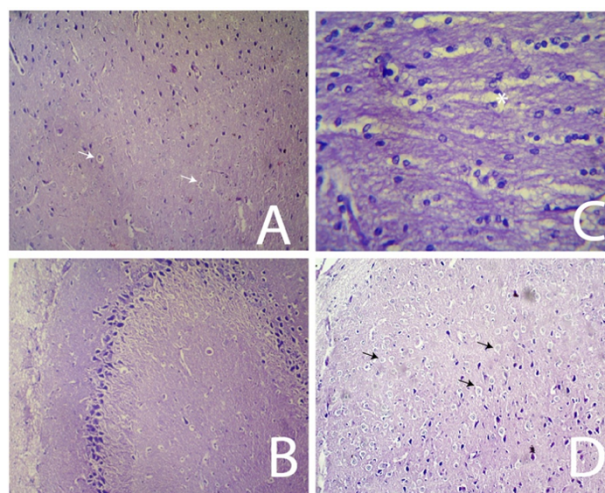


Figure 2: Photomicrograph of levodopa and benserazide treated rat brain showing vacuolations (white arrows) in the cerebrum (A), normal cerebellum (B), edema (*) in the striatum (C) and pyknotic and hyperchromatic nuclei (black arrows) in the thalamus (D)

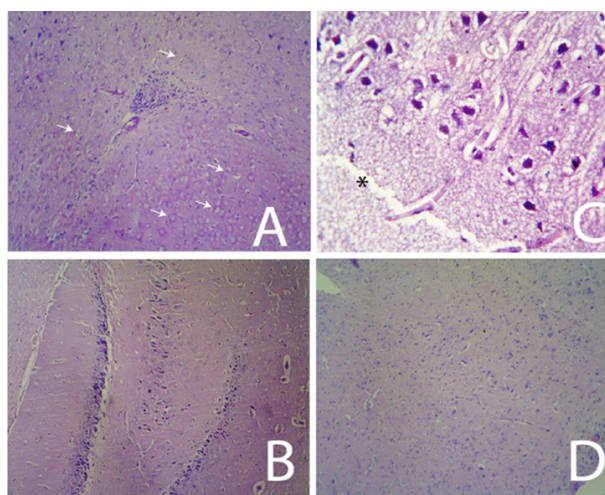


Figure 3: Photomicrograph of captopril treated rat brain showing vacuolations (white arrows) around the cerebrum (A), normal cerebellum (B), edema (*) in the striatum (C) and normal thalamus (D)

In the present study, behavioral effects were studied with rotarod test and spontaneous locomotor activity, as evident through the actophotometer. These two methods are well-established means of measuring overall behavioral changes in the rodent model. The length of time (duration) the animal stays on the rod without falling gives a measure of their coordination, balance, physical condition, and motor-planning. Rotarod test is a commonly used test to evaluate the beneficial effects of the test drugs and molecules. (Carvalho *et al.*, 2013) Captopril has shown significant improvement in motor coordina-

Table 4: Tabulation of oxidative stress markers in the rotenone model

	Control group	Rotenone group	Rotenone + levodopa group	Rotenone + Captopril group
GSH	8.1 ± 1.5	4.8 ± 2.1*	6.3 ± 0.7†	5.3 ± 2.1*
LPO	15.04 ± 1.4	36.6 ± 7.1†	27.5 ± 2.9†	25.8 ± 6.1*
MPO	60.4 ± 1.0	139.7 ± 1.6†	87.97 ± 0.9†	106.1 ± 4.9†
Catalase	5.88 ± 0.05	3.56 ± 0.08†	6.54 ± 0.17†	8.21 ± 0.04†
SOD	3.28 ± 0.08	2.08 ± 0.01*	8.54 ± 1.02†	6.4 ± 0.04†
MAO-A	1.2 ± 0.02	1.08 ± 0.1†	1.12 ± 0.7	0.89 ± 0.7†
MAO-B	1.1 ± 0.06	0.8 ± 0.5†	1.1 ± 0.1	1.5 ± 0.2†

Glutathione(GSH, oxidised/min/g protein), Lipid peroxidation (LPO, nmol of MDA/mgprotein), myeloperoxidase (MPO, μ mol/min/mg tissue), catalase (μ moles of H₂O₂ used/min/mgprotein), Superoxide dismutase (SOD, units/mg protein) and monoamineoxidase (MAO-A and MAO-B, u/mg protein) from each group (n=6) expressed as mean \pm standard deviation, †p<0.001,*p<0.05 in comparison to control group

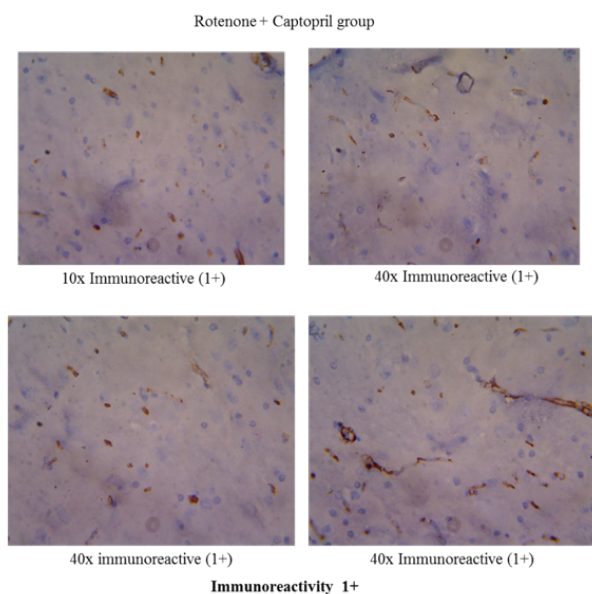


Figure 4: Immunohistochemistry of the midbrain region of control group showing 1+ immunoreactivity in the rotenone model, rotenone + captopril group

tion, as evidenced through the rotarod test. Similarly improved motor co-ordination preventing the rat or mice from falling from the rotarod has been recorded after the administration of *Dendropanax moribiferus* leaves (Park et al., 2018)

Apium graveolens (Chonpathompikunlert et al., 2018) (MPTP model), *Juniperus communis* (Bais et al., 2015) (CPZ model), caffeine (Khadrawy et al., 2017) (rotenone model) and with many other chine herbal medicines. (Kum et al., 2011; Pan et al., 2011) However, in the present study, captopril has not shown significant improvement in spontaneous locomotor activity in the rotenone model. Contrary to our finding, improvement in sponta-

neous locomotor activity was observed after administration of *Juniperus communis* (Bais et al., 2015) (in chlorpromazine induced rat model), *Trigonella foenum-graecum* seed extract (Gaur et al., 2013) (in 6-OHDA induced unilateral PD in rats) and in *Artemisia* flowers (Pal and Ghosh, 2018) (in chlorpromazine induced rat model) and curcumin and derivatives (Agrawal et al., 2012) (in 6-OHDA induced rat model). In the present study, captopril had shown significant improvement in exploratory behavior in the rotenone model. Hole board test has been successfully used to determine the beneficial effects of caffeine (Khadrawy et al., 2017) arbutin (Dadgar et al., 2018) dasatinib and resveratrol. (T. and R., 2019) Most commonly, behavioral despair is evaluated with a forced swimming test. In the present study, captopril had shown significantly improved performance in forced swim tests in the rotenone model. Forced swimming test has been successfully used to evaluate the antidepressant activity of imipramine (Gutiérrez-García and Contreras, 2009), fluoxetine, reboxetine, moclobemide (Cryan et al., 2005) and nitroindazole (Yildiz et al., 2000). It is proven that serotonin-selective reuptake inhibitors (fluoxetine, certraline, sertraline) increase swimming behavior. Drugs that increase norepinephrine and dopamine improves climbing behaviour (Page et al., 1999)

The hallmark of PD is the loss of dopaminergic neurons in substantia nigra pars compacta. Efficacies of the animal models are judged based on the degree to which it results in this neuronal loss leading to dopamine depletion. There are many methods to quantify neuronal loss. One of the broader methods is to quantify the dopamine levels in the brain (Eschlböck et al., 2015). Recorded dopamine levels in brain homogenates from the

captopril group of the rotenone model showed a significant increase in dopamine. This may be attributed to neuroprotection and less severe damage to dopaminergic neurons among these rats. Similar improvement in dopamine levels postulated to be due to neuroprotective effects of test drugs has been reported after administration of biochanin A (Yu *et al.*, 2017) (in MPTP model), adenosine A receptor blockers (Fathalla *et al.*, 2016) (rotenone model) and catechin (Teixeira *et al.*, 2013) (6-OHDA model). There are many reports concentrating on the dopamine levels in the specific regions of the brain and has shown that co-administration of artemisi (Pal and Ghosh, 2018), Juniperus (Bais *et al.*, 2015) and much Chinese herbal preparation (Kum *et al.*, 2011; Pan *et al.*, 2011) resulted in improved dopamine levels. This concludes that these interventions result in lesser neuronal damage.

Serotonin levels were significantly reduced in the captopril pre-treated group. This, along with a documented reduction in the bradykinesia, indicates that serotonergic pathways contribute to the overall effects of levodopa and captopril. However, the exact mechanism of action can not be ascertained by the estimation of total serotonin (Hawkes *et al.*, 2009). There seems to be a complex interplay between serotonergic neurons and the release of dopamine, as evidenced in a study on levodopa-induced dyskinesia in rats. (Carta *et al.*, 2007; Shin *et al.*, 2012)

In the present study, GABA levels were significantly lower in the captopril group of the rotenone model. GABA is an important inhibitory neurotransmitter; lower levels of GABA with an increase in calcium has been the proven pathway of neuronal destruction not just in PD but also in Alzheimer's disease. (Pal and Ghosh, 2018)

The role of oxidative stress in neuroinflammatory processes has been extensively evaluated with various drugs and plant products. (Wei *et al.*, 2018) Most of the studies advocate a direct relationship of free radicals with microglia, astrocytes, and neurons in this process. (Peterson and Flood, 2012)

In the present study, oxidized glutathione levels were significantly lower in captopril pretreated rats in rotenone model rats. Similarly, the changes in lipid peroxidase, myeloperoxidase, catalase, superoxide dismutase, and MAO-B indicates effective and significant scavenging of free radical species during and after toxin-induced neuronal damage in captopril pre-treated animals. Similar neuroprotective roles of some of the herbal derivatives from ginger (curcumin), ginseng (ginsenoside), and polygonum

cuspidatum (resveratrol) have been reported. (Fu *et al.*, 2015) Herbal extracts like the moutan cortex, Angelica dahurica root, and bupleurum root also exerts neuroprotective action in PD. (Jeong *et al.*, 2018) Many flavonoids are proposed to exhibit neuroprotective actions primarily through anti-oxidant mechanisms. (Magalingam *et al.*, 2015) Apart from these, artemisia (Pal and Ghosh, 2018), isolongifolene (Balakrishnan *et al.*, 2018), and caffeine (Khadrawy *et al.*, 2017) and many other drugs have shown significant therapeutic roles in PD.

The brain section of captopril treated rodents showed histopathological lesions like vacuolations (in cerebrum, cerebellum, striatum, thalamus), cerebellar cortical changes (apoptotic cells, astrocyte variations, pale granular cells), glial pale bodies, edema (in cerebrum and substantia nigra), hyperchromatic and pyknotic nuclei in specific regions of the brain. However, the overall neuronal architecture was not altered significantly in comparison to the rodent brains that were not treated with these drugs. The histological picture is in line with the neurobehavior effects, neurotransmitter levels, and oxidative stress marker levels in protecting the neurons against the rotenone-induced toxicity.

Immunohistochemistry of Bcl-2 is a strong indicator of anti-apoptosis. Figure 4 Immunohistochemical studies have shown that Bcl-2 is normally expressed in the limbic cortex, hippocampus, and cerebellar cortex. (M. *et al.*, 2019) The scoring system used in the present study is in line with the new criteria defined by Tsuyama *et al.* for Bcl-2 quantification in blood dyscrasias, especially B-cell related leukaemia. (Tsuyama *et al.*, 2017) Downregulation of Bcl-2 has been reported in the 6-OHDA model rat Parkinsonism. (Xu *et al.*, 2015) Studies have quantified the beneficial effects of scorpion venom derived activity peptide (Xu *et al.*, 2015) and Shudipingchan granules (Ye *et al.*, 2016) in a rat model of Parkinsonism in terms of a significant increase in Bcl-2 immunohistochemical reactivity. In the present study, the captopril group has shown a mild (1+) immunoreactivity to Bcl2.

Limitations of the study: Estimation of dopamine from the midbrain sections alone would have resulted in more accurate effects of captopril on the substantia nigra. Neurotransmitter estimation is a crude way of assessing overall effects. These methods may not delineate the synaptic, presynaptic, and dendritic, astrocyte-specific concentrations, specific effects, and differential outcomes. Quantification of receptors (by autoradiography) would have resulted in a better understanding of the specific neuroprotective role of these investigational drugs in PD. Use

of immunohistochemistry to delineate the formation and localization of α -synuclein and Lewy bodies would have significantly added to the outcome of the study.

CONCLUSIONS

Captopril has shown significant improvement in motor coordination (as evidenced through rotarod test), exploratory behavior (hole board test), depression (forced swimming test, and tail suspension test). Captopril has significant effects on brain neurotransmitters, as evidenced by dopamine, serotonin, and acetylcholine. Captopril has a significant neuroprotective role, as evidenced by changes in glutathione, lipid peroxidase, myeloperoxidase, catalase, superoxide dismutase, and MAO-B. Captopril has not shown major histoanatomical changes in the rotenone model. Captopril has shown significant neuronal protection by increased expression of Bcl-2 immunohistochemistry in rotenone-induced PD.

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Conflict of Interest

Authors declare no conflict of interest

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REFERENCES

- Agrawal, S. S., Gullaiya, S., Dubey, V., Singh, V., Kumar, A., Nagar, A., Tiwari, P. 2012. Neurodegenerative Shielding by Curcumin and Its Derivatives on Brain Lesions Induced by 6-OHDA Model of Parkinson's Disease in Albino Wistar Rats. *Cardiovasc. Psychiatry Neurol.*
- Ambhore, N. S., Prasanna, M., Antony, A. S., Kumar, M. S., Elango, K. 2014. Pharmacological and antioxidant evaluation of Aspirin, nimodipine, and its combination for anti-Parkinson's activity in MPTP induced rat model. *Int. J. Health Allied Sci*, 3.
- Bais, S., Gill, N. S., Kumar, N. 2015. Neuroprotective Effect of Juniperus communis on Chlorpromazine Induced Parkinson Disease in Animal Model. *Chin. J. Biol.*, pages 542542–542542.
- Balakrishnan, R., Elangovan, N., Mohankumar, T., Nataraj, J., Manivasagam, T., Thenmozhi, A. J., Essa, M. M., Akbar, M., Khan, M. A. S. 2018. Isolongifolene attenuates rotenone-induced mitochondrial dysfunction, oxidative stress, and apoptosis. *Front. Biosci. Sch. Ed*, 10:248–261.
- Bhangale, J. O., Acharya, S. R. 2016. Anti-Parkinson Activity of Petroleum Ether Extract of Ficus religiosa (L.) Leaves. *Adv. Pharmacol. Sci*, 9436106.
- Brown, G. R., Nemes, C. 2008. The exploratory behavior of rats in the hole-board apparatus: Is head-dipping a valid measure of neophilia? *Behav. Processes*, 78:442–448.
- Can, A., Dao, D. T., Arad, M., Terrillion, C. E., Piantadosi, S. C., Gould, T. D. 2012a. The Mouse Forced Swim Test. *J. Vis. Exp. JoVE*, 59.
- Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., Gould, T. D. 2012b. The Tail Suspension Test. *J. Vis. Exp. JoVE*, 59.
- Carta, M., Carlsson, T., Kirik, D., Björklund, A. 2007. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain J. Neurol*, 130:1819–1833.
- Carvalho, M. M., Campos, F. L., Coimbra, B., Pêgo, J. M., Rodrigues, C., Lima, R., Rodrigues, A. J., Sousa, N., Salgado, A. J. 2013. Behavioral characterization of the 6-hydroxy dopamine model of Parkinson's disease and pharmacological rescuing of non-motor deficits. *Mol. Neurodegener*, 8.
- Chonpathompikunlert, P., Boonruamkaew, P., Sukketsiri, W., Hutamekalin, P., Sroyraya, M. 2018. The antioxidant and neurochemical activity of Apium graveolens L. and its ameliorative effect on MPTP-induced Parkinson-like symptoms in mice. *BMC Complement. Altern. Med*, 18:103–103.
- Cryan, J. F., Page, M. E., Lucki, I. 2005. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology (Berl.)*, 182:335–344.
- Dadgar, M., Pouramir, M., Dastan, Z., Ghasemi-Kasman, M., Ashrafpour, M., Moghadamnia, A. A., Khafri, S., Pourghasem, M. 2018. Arbutin attenuates behavioral impairment and oxidative stress in an animal model of Parkinson's disease. *Avicenna J. Phytomedicine*, 8:533–542.
- Deacon, R. M. J. 2013. Measuring Motor Coordination in Mice. *J. Vis. Exp. JoVE* e2609.
- Devasagayam, T. P. A., Bloor, K. K., Ramasarma, T. 2003. Methods for estimating lipid peroxidation: an analysis of merits and demerits. *Indian J. Biochem. Biophys*, 40:300–308.
- Eschlböck, S., Krismer, F., Wenning, G. K. 2015. Interventional trials in atypical parkinsonism. In *Proceedings of XXI World Congress on Parkinson's Dis-*

- ease and Related Disorders*, volume 22, pages 82–92.
- Fathalla, A. M., Soliman, A. M., Ali, M. H., Moustafa, A. A. 2016. Adenosine A2A Receptor Blockade Prevents Rotenone-Induced Motor Impairment in a Rat Model of Parkinsonism. *Front. Behav. Neurosci*, 10.
- Fu, W., Zhuang, W., Zhou, S., Wang, X. 2015. Plant-derived neuroprotective agents in Parkinson's disease. *Am. J. Transl. Res*, 7:1189–1202.
- Gaur, V., Bodhankar, S. L., Mohan, V., Thakurdesai, P. A. 2013. Neurobehavioral assessment of hydroalcoholic extract of *Trigonella foenum-graecum* seeds in rodent models of Parkinson's disease. *Pharm. Biol*, 51:550–557.
- Gutiérrez-García, A. G., Contreras, C. M. 2009. Stressors can affect immobility time, and response to imipramine in the rat forced swim test. *Pharmacol. Biochem. Behav*, 91:542–548.
- Hawkes, C. H., Tredici, K. D., Braak, H. 2009. Parkinson's Disease, The Dual Hit Theory Revisited. *Ann. N. Y. Acad. Sci*, 1170:615–622.
- Hellner, K., Walther, T., Schubert, M., Albrecht, D. 2005. Angiotensin-(1-7) enhances LTP in the hippocampus through the G-protein-coupled receptor Mas. *Mol. Cell. Neurosci*, 29:427–435.
- Jeong, J. S., Piao, Y., Kang, S., Son, M., Kang, Y. C., Du, X. F., Ryu, J., Cho, Y. W., Jiang, H. H., Oh, M. S., Hong, S. P., Oh, Y. J., Pak, Y. K. 2018. Triple herbal extract DA-9805 exerts a neuroprotective effect via amelioration of mitochondrial damage in experimental models of Parkinson's disease. *Sci. Rep*, 8:1–13.
- Kerr, D. S., Bevilacqua, L. R. M., Bonini, J. S., Rossato, J. I., Köhler, C. A., Medina, J. H., Izquierdo, I., Cammarota, M. 2005. Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. *Psychopharmacology (Berl.)*, 179:529–535.
- Khadrawy, Y. A., Salem, A. M., El-Shamy, K. A., Ahmed, E. K., Fadl, N. N., Hosny, E. N. 2017. Neuroprotective and Therapeutic Effect of Caffeine on the Rat Model of Parkinson's Disease Induced by Rotenone. *J. Diet. Suppl*, 14:553–572.
- Kum, W. F., Durairajan, S. S. K., Bian, Z. X., Man, S. C., Lam, Y. C., Xie, L. X., Lu, J. H., Wang, Y., Huang, X. Z., Li, M. 2011. Treatment of idiopathic Parkinson's disease with traditional Chinese herbal medicine: a randomized placebo-controlled pilot clinical study. *Evid.-Based Complement. Altern. Med. ECAM*.
- Kurosaki, R., Muramatsu, Y., Imai, Y., Kato, H., Araki, T. 2004. Neuroprotective effect of the angiotensin-converting enzyme inhibitor perindopril in MPTP-treated mice. *Neurol. Res*, 26:644–657.
- Lee, Y. C., Lin, C. H., Wu, R. M., Lin, J. W., Chang, C. H., Lai, M. S. 2014. Antihypertensive agents and risk of Parkinson's disease: a nationwide cohort study. *PloS One*, 9.
- M., J., Muriach, B. G., Miranda, M., F, FJ, B., R. 2019. Localization of BCL-2 in the mouse brain: An anatomical study by immunohistochemistry. *Eur. J. Anat*, 11:69–76.
- Magalingam, K. B., Radhakrishnan, A. K., Haleagrahara, N. 2015. Protective Mechanisms of Flavonoids in Parkinson's Disease. *Oxid. Med. Cell. Longev*, pages 314560–314560.
- Malusecka, E., Zborek, A., Krzyzowska-Gruca, S., Krawczyk, Z. 2006. Immunohistochemical Detection of the Inducible Heat Shock Protein Hsp70: A Methodological Study. *J. Histochem. Cytochem*, 54:183–190.
- Maul, B., Krause, W., Pankow, K., Becker, M., Gembarth, F., Alenina, N., Walther, T., Bader, M., Siems, W. E. 2005. Central angiotensin II controls alcohol consumption via its AT1 receptor. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol*, 19:1474–1481.
- Muñoz, A., Rey, P., Guerra, M. J., Mendez-Alvarez, E., Soto-Otero, R., Labandeira-Garcia, J. L. 2006. Reduction of dopaminergic degeneration and oxidative stress by inhibition of the angiotensin-converting enzyme in an MPTP model of parkinsonism. *Neuropharmacology*, 51:112–120.
- O'callaghan, J. P., Imai, H., Miller, D. B., Minter, A. 1999. Quantitative immunoblots of proteins resolved from brain homogenates: underestimation of specific protein concentration and of treatment effects. *Anal. Biochem*, 274:18–26.
- Page, M. E., Detke, M. J., Dalvi, A., Kirby, L. G., Lucki, I. 1999. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology (Berl.)*, 147:162–167.
- Pal, P., Ghosh, A. K. 2018. Antioxidant, Anti-Alzheimer, and Anti-Parkinson Activity of *Artemisia nilagirica* Leaves with Flowering Tops. *UK J. Pharm. Biosci*, 6.
- Pan, W., Kwak, S., Liu, Y., Sun, Y., Fang, Z., Qin, B., Yamamoto, Y. 2011. Traditional Chinese medicine improves activities of daily living in Parkinson's disease. *Park. Dis*.
- Park, S. Y., Karthivashan, G., Ko, H. M., Cho, D. Y., Kim, J., Cho, D. J., Ganesan, P., Su-Kim, I., Choi, D. K. 2018. Aqueous Extract of *Dendropanax morbiferus* Leaves Effectively Alleviated Neuroinflammation and Behavioral Impediments in MPTP-Induced Parkinson's Mouse Model. *Oxid. Med. Cell*.

- Longev, 14.
- Peng, J., Kimura, B., Phillips, M. I. 2002. The predominant role of brain angiotensinogen and angiotensin in environmentally induced hypertension. *Regul. Pept*, 110:25–32.
- Peterson, L. J., Flood, P. M. 2012. Oxidative Stress and Microglial Cells in Parkinson's Disease. *Mediators Inflamm*.
- Pulli, B., Ali, M., Forghani, R., Schob, S., Hsieh, K. L. C., Wojtkiewicz, G., Linnoila, J. J., Chen, J. W. 2013. Measuring Myeloperoxidase Activity in Biological Samples. *PLoS ONE*, 8.
- Reardon, K. A., Mendelsohn, F. A., Chai, S. Y., Horne, M. K. 2000. The angiotensin-converting enzyme (ACE) inhibitor, perindopril, modifies the clinical features of Parkinson's disease. *Aust. N. Z. J. Med*, 30:48–53.
- Rees, K., Stowe, R., Patel, S., Ives, N., Breen, K., Ben-Shlomo, Y., Clarke, C. E. 2011. Anti-hypertensive drugs as disease-modifying agents for Parkinson's disease: evidence from observational studies and clinical trials. *Cochrane Database Syst. Rev*, 9.
- Saab, Y. B., Gard, P. R., Yeoman, M. S., Mfarrej, B., El-Moalem, H., Ingram, M. J. 2007. Renin-angiotensin-system gene polymorphisms and depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 31:1113–1118.
- Sgroi, S., Kaelin-Lang, A., Capper-Loup, C. 2014. Spontaneous locomotor activity and L-DOPA-induced dyskinesia are not linked in 6-OHDA parkinsonian rats. *Front. Behav. Neurosci*, 8:331–331.
- Shin, E., Tronci, E., Carta, M. 2012. Role of Serotonin Neurons in L-DOPA- and Graft-Induced Dyskinesia in a Rat Model of Parkinson's Disease. *Park. Dis*. 370190.
- Sonsalla, P. K., Coleman, C., Wong, L. Y., Harris, S. L., Richardson, J. R., Gadad, B. S., Li, W., German, D. C. 2013. The angiotensin-converting enzyme inhibitor captopril protects nigrostriatal dopamine neurons in animal models of parkinsonism. *Exp. Neurol*, 250:376–383.
- T., A. N., R., V. 2019. Behavioral studies of dasatinib and resveratrol in rotenone-induced Parkinson's rat model. *Int J Pharm Sci Res*, 10:200–211.
- Teixeira, M. D. A., Souza, C. M., Menezes, A. P. F., Carmo, M. R. S., Fonteles, A. A., Gurgel, J. P., Lima, F. A. V., Viana, G. S. B., Andrade, G. M. 2013. Catechin attenuates behavioral neurotoxicity induced by 6-OHDA in rats. *Pharmacol. Biochem. Behav*, 110:1–7.
- Tipple, T. E., Rogers, L. K. 2012. Methods for the Determination of Plasma or Tissue Glutathione Levels. *Methods Mol. Biol. Clifton, NJ*, 889:315–324.
- Tsuyama, N., Sakata, S., Baba, S., Mishima, Y., Nishimura, N., Ueda, K., Yokoyama, M., Terui, Y., Hatake, K., Kitagawa, M., Ishizuka, N., Tomita, N., Takeuchi, K. 2017. BCL2 expression in DLBCL: a reappraisal of immunohistochemistry with new criteria for therapeutic biomarker evaluation. *Blood*, 130:489–500.
- Wei, Z., Li, Xiaowan, Li, Xixi, Liu, Q., Cheng, Y. 2018. Oxidative Stress in Parkinson's Disease: A Systematic Review and Meta-Analysis. *Front. Mol. Neurosci*, 11.
- Wright, J. W., Harding, J. W. 2012. Importance of the Brain Angiotensin System in Parkinson's Disease. *Park. Dis*. 860923.
- Xu, H., An, D., Yin, S., Chen, W., Zhao, D., Meng, X., Yu, D., Sun, Y., Zhao, J., Zhang, W. 2015. The alterations of apoptosis factor Bcl-2/Bax in the early Parkinson's disease rats and the protective effect of scorpion venom derived activity peptide. *Zhongguo Ying Yong Sheng Li Xue Za Zhi Zhongguo Yingyong Shenglixue Zazhi Chin. J. Appl. Physiol*, 31:225–229.
- Yamamoto, B. J., Elias, P. D., Masino, J. A., Hudson, B. D., Mccoy, A. T., Anderson, Z. J., Varnum, M. D., Sardinia, M. F., Wright, J. W., Harding, J. W. 2010. The Angiotensin IV Analog Nle-Tyr-Leu-ψ-(CH₂-NH₂)₃-4-His-Pro-Phe (Norleual) Can Act as a Hepatocyte Growth Factor/c-Met Inhibitor. *J. Pharmacol. Exp. Ther*, 333:161–173.
- Ye, Q., Yuan, X., He, J., Zhou, J., Yuan, C., Yang, X. 2016. Anti-apoptotic effect of Shudipingchan granule in the substantia nigra of rat models of Parkinson's disease. *Neural Regen. Res*, 11:1625–1632.
- Yildiz, F., Erden, B. F., Ulak, G., Utkan, T., Gacar, N. 2000. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. *Psychopharmacology (Berl.)*, 149:41–44.
- Yu, L., Wang, X., Chen, H., Yan, Z., Wang, M., Li, Y. 2017. Neurochemical and Behavior Deficits in Rats with Iron and Rotenone Co-treatment: Role of Redox Imbalance and Neuroprotection by Biochanin A. *Front. Neurosci*, 11.
- Yu, Z. F., Kong, L. D., Chen, Y. 2002. Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. *J. Ethnopharmacol*, 83:161–165.