CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Online at: http://www.iajps.com *Research Article*

NEUROPROTECTIVE ACTIVITY OF *CARISSA CARANDAS* ROOT AGAINST EXCITOTOXICITY MODEL OF PARKINSON'S DISEASE IN RODENTS AND ZEBRAFISH

Veronica Belchir Pereira*[1] and Rachana D. Sarawade[2]

[1]PG Student, Dr L H Hiranandani College of Pharmacy, Pharmacology Department, Ulhasnagar-03

[2]Assistant Professor/H.O. D in Dr L H Hiranandani college of pharmacy, Pharmacology Department, Ulhasnagar-03

Article Received: August 2021 **Accepted:** August 2021 **Published:** September 2021

Abstract:

Background: Herbal plants or compounds from plant are used all over the world to treat different diseases. On of the advantage of using plant origin compound is negligible or lesser side effect. Carissa carandas is well known plant used for many disorders including brain disorders. Though having many bioactive compounds its affect in Parkinson's disease is unknown. Hence neuroprotective effect of Carissa carandas was explored in this study.

***Methodology:** Acute toxicity study was first performed in zebrafish. Parkinson's disease was induced by Rotenone and behavioral parameters were evaluated on 3rd day. Excitotoxicity was then induced using monosodium glutamate (MSG) in Wistar rats for 7days. On 8th day, readings of locomotor activity, neuromuscular strength, motor rigidity were recorded and on 9th day, biochemical parameters i.e., levels of dopamine, catalase and glutathione were analyzed using UV-Vis spectrophotometer. **Results:** The maximum tolerated extract dose was 100mg/L in zebrafish. The EECC was found to be neuroprotective as it reduces symptoms of Parkinson disease. This activity may be due to the ability of EECC to antagonize NMDA receptor and decrease the elevated Calcium level. **Conclusion:** An Ethanolic extract of Carissa carandas root showed promising effect in improving symptoms of Parkinson's disease and hence can be used as a neuroprotective agent in near future.*

***Keywords**: Carissa carandas, Excitotoxicity, NMDA, Calcium, Neuroprotective agent*

Corresponding author:

Veronica Belchir Pereira*,

PG Student,

Dr L H Hiranandani College of Pharmacy,

Pharmacology Department, Ulhasnagar-03

veronica.pereira@dlhhcop.org

9769289709

QR code

Please cite this article in press Veronica Belchir Pereira et al., ***Neuroprotective Activity Of Carissa Carandas Root Against Excitotoxicity Model Of Parkinson's Disease In Rodents And Zebrafish****.., Indo Am. J. P. Sci, 2021; 08(09).*

INTRODUCTION:

Neurodegeneration is a characteristic of many age-related and incurable diseases that are rapidly rising in prevalence, such as Parkinson's disease. There is an urgent need to develop new and more effective therapeutic strategies to combat these calamitous diseases. Over the years this neurogenerative disease are increasing and specifically affecting elderly population.Effective treatments are desperately needed but will only come with a deep understanding of the causes and mechanisms of each disease.[1]

Parkinson's disease is the second most common age-related - progressive neurodegenerative disorder of the extrapyramidal motor system characterized by loss of dopaminergic neurons in substantia nigra pars compacta of basal ganglia.[2] An estimated seven to 10 million people worldwide have Parkinson's disease, being present in 1% of people over the age of 65 years.The motor symptoms of PD are attributed to the loss of striatal dopaminergic neurons, although the presence of nonmotor symptoms supports neuronal loss in nondopaminergic areas as well. The term *parkinsonism* is a symptom complex used to describe the motor features of PD, which include resting tremor, bradykinesia, and muscular rigidity. PD is the most common cause of parkinsonism, although a number of secondary causes also exist, including diseases that mimic PD and drug-induced causes.[3]Enhanced level of glutamate levels causes excessive stimulation of NMDA receptor which initiates various pathways that leads to neuronal death. NMDA hyperactivation leads to a pathological process called excitotoxicity and protein aggregation and misfolding. Excitotoxicity is due to an increase in Ca2+levels.[2]

Herbal plants have long been known all over the world for their remedial benefits. This plants are known to increase the standard of health specially in rural areas. With different bioactive ingredients present in herbal plants, make them a attractive area for research. Scientists all over world are finding different strategies for using herbal plants in treatment for better ment of the patients. Many bioactive compounds present in plants can cross the blood brain barrier making them suitable for treating neurodegenerative diseases.The current treatment of PD have some side effects,using plant origin compounds may have lesser effect hence,the demand for new oral medicines without side effects continues.[4]

Carissa carandas possess anticonvulsant, antioxidant activity. Ethanolic extract of root has been known to protect against NMDA induced convulsion and significantly prolonged the latency of the seizures. It contains various constituent which have neuroprotective activity such as Triterpene derivatives - α-Amyrin, Oleanolic acid, Ursolic acid, Lupeol, Phytosterols-β-Sitosterol.[5] Hence there is probability that it could act as neuroprotective against excitotoxicity model of Parkinson's disease by antagonizing NMDA receptor, whose overstimulation leads to excitotoxicity. Its neuroprotective activity against Parkinson's disease is yet to be explored. Hence the aim is to explore its activity against excitotoxicity model of Parkinson's disease. Thus, with this present study an attempt to evaluate neuroprotective activity against excitotoxicity model of Parkinson's disease.

MATERIALS AND METHODS:

Drugs and Chemicals *Carissa carandas* root powder (Dhanvantry Aushadhi Bhandar, Vasai West). Monosodium glutamate (MSG) was purchased from Himedia Lab., Selegiline- (Themis medicare Ltd.), Rotenone- (Sigma Aldrich), Dopamine (Sigma Aldrich), 5,5-dithiobis[2- nitrobenzoic acid] (Sigma Aldrich).

Animals

Adult wild type zebrafish, 0.5-1g were procured from Vikrant aqua culture, Bandra and the Wistar rats, 200-250g were procured, from National Institute of Biosciences, Pune. The zebrafish was brought and acclimatized in Zebrafish facility located in M. Pharm Pharmacology laboratory of Dr. L.H. Hiranandani college of pharmacy, opposite to Ulhasnagar railway station, CHM campus, Ulhasnagar-03 under standard husbandry conditions, i.e., temperature of 28 ± 5ºC, optimum pH of 7-8, conductivity of 0.25ppt-0.75ppt and 14:10 hr. light/dark cycle and the rats were brought to animal house of Dr. L.H. Hiranandani college of pharmacy, opposite to Ulhasnagar railway station, CHM campus, Ulhasnagar-03. Rats were acclimatized in animal house under standard husbandry conditions, i.e. room temperature of 24 ± 10ºC, relative humidity 45-55% and 12:12 hr. light/dark cycle and. The institutions animal house is registered with Govt. of India, having registration number 879/PO/Re/S/05/CPCSEA and confirms to the CPCSEA guidelines for the use and care of experimental animal research. The animals were housed in standard propylene cages with wire mesh top and husk as bedding. The animals had free access for food and water supplied ad libitum under strict hygienic condition. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. The approval of the of the Institutional

animal ethical committee (IAEC) of Dr. L.H. Hiranandani College of pharmacy was taken prior to the start of experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSEA) and with the protocol no. PCOL/IAEC/2019/10 (for rats) and PZEB/IAEC/2019/08 (for zebrafish).

Preparation of EECC extract

Root powder (100 g) was extracted with 50% ethanol by cold maceration for three days with occasional shaking. The solvent from the total extract was filtered & concentrated on water bath, to get semisolid product. The Concentrated Ethanolic Extract was placed in Hot air oven to get dry powder of the extract & this extract powder was stored in a refrigerator and used for further studies.When needed the extract was dissolved in distilled water and used.[6] The observed yield was 4%.

Physicochemical, Preliminary Phytochemical analysis and Confirmatory test

Physicochemical analysis of crude extract was performed, parameters such as total ash value, acid insoluble and water-soluble ash value and loss on drying was performed.7

Preliminary phytochemical screening was done for the presence of different group of chemicals, that is, alkaloids, flavonoids, saponins, tannins, diterpenes, carbohydrates.

Confirmatory test of Triterpenoids was performed to confirm the presence of triterpenoids.

Acute toxicity study

Zebrafish- Acute toxicity was performed on test compound (EECC) according to OECD 203 in 8 zebrafish**.** The fishes were exposed to the EECC at a maximum dose of 100mg/L for a period of 96 hours. Mortalities were recorded at 24, 48, 72 & 96 hours.
Parameters evaluated were Number and duration of freezing episodes, Complete cataleptic time ,Time spent near the bottom of tank , Latency to travel from one point to another ,Total distance moved and swimming velocity. The fate of zebrafish was rehabilitation.

Rats-

Acute toxicity for plant root was earlier carried out according to OECD guideline 425.[5,9,10].The highest dose used for testing was 2000mg/kg p.o. There was no mortality found in animals and they did not show any toxicity or behavioural changes. It was considered nontoxic.[10] So was not performed.

***In vivo* studies:**

In Zebrafish: To evaluate the neuroprotective effect of EECC in Rotenone induced Parkinson's disease in Zebrafish.

The experimental group consists of 40 adult wildtype zebrafishes divided in 5 groups of 8 zebrafish in each group. The study was carried out for a period of 72 hrs. First group was administered with 10%DMSO, second group with Rotenone (3pg/ml), third, fourth and fifth group was first administered with Rotenone and then administered with standard Selegiline 0.03µg/ml, Test 1 -5µg/ml (EECC), Test 2- 5µg/ml (EECC) respectively.

All fishes were exposed to Rotenone at a dose of 3pg/ml for 30 min. After inducing Parkinson's disease fishes were transferred to fresh aerated water for 15 min. Later fishes were exposed to EECC or Standard drug according to the groups for 30min and then in fresh aerated water for 15min. This schedule was followed for 3 days and on the 3rd day, fishes were placed in Experimentation tank.

Experimentation tank was filled up to 5L fresh aerated tank water. The tank was marked with horizontal and vertical lines for easy evaluation of behavioural parameters. Number and duration of freezing episodes ,Complete cataleptic time ,Time spent near the bottom of tank , Latency to travel from one point to another ,Total distance moved and swimming velocity. All behavioural evaluation was done using camera.[11]

In Rats: To evaluate the neuroprotective effect of EECC in MSG induced excitotoxicity in rats.

The experimental group consists of 30 wistar rats. The study was carried out for a period of 7 days. These rats were randomized and divided into 5 groups, where each group consists of 6 rats. First group was treated with vehicle i.e. 1%CMC at a dose of 5ml/kg peroral administration. Except vehicle group, all the remaining four groups was treated with MSG at a dose of 2000mg/kg i.p. In third group, after 1 hr treatment of MSG, standard i.e. Selegiline was treated at a dose of 30mg/kg peroral administration. In fourth and fifth group, EECC was treated at a dose of 400mg/kg and 1000mg/kg per oral administration following one hour treatment of MSG administration.[12]

On 8th day, Behavioural parameters such as Locomotor activity by actophotometer[12,] Motor rigidity by horizontal bar test[13, Neuromuscular] strength will

be observed by hang test On 9th day, rats of all the groups were sacrificed by exposure to CO2 chamber and brains were dissected, and dipped in Tris buffer solution. Brain was homogenized with a ratio of 1:15 (1g brain: 15 ml Tris buffer solution) [12], in handheld homogenizer at a speed of 5000rpm for 10min to get a brain homogenate. Brain homogenate obtained was subjected to cooling refrigerated centrifuge machine at 12000rpm for 5 min to get sediment and supernatant. The sediment was discarded and supernatant was collected and stored at 4°C and biochemical parameters such as Glutathione ,Dopamine and Catalase were analyzed.[12]

Estimation of Glutathione:
Brain tissue homogenate was centrifuged at 16,000 g for 15-minute 4◦C.The supernatant (0.5 ml) was added to 4 ml of ice-cold 0.1 Mm solution of 5, 5-dithiobis [2-nitrobenzoic acid] in 1 M phosphate buffer (pH 8). The optical density was read at 412 nm in a spectrophotometer.[14]

Estimation of Dopamine:
About 20mg of pure dopamine was accurately weighed and dissolved in 20ml distilled water to form 1000ppm (1000µg/ml) concentration. An aliquot of 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the drug solution was transferred into a series of 25 ml standard volumetric flasks to form 8, 16, 24, 32 and 40ppm (µg/ml) concentration. To each flask, 1 ml of 4N hydrochloric acid and 1 ml of 0.02N brominating mixture were added. The flasks were shaken well and set aside for 5min for complete bromination. Then, 1ml of 0.1N potassium iodide was added to each flask and diluted to 25 ml with distilled water. The yellow solution formed was measured at 280nm against distilled water as a blank. A calibration curve was obtained by plotting Concentration on X axis and Absorbance on Y axis. 1ml of Supernatant of rat brain was transferred to 25ml flask. To the flask, 1ml of 4N hydrochloric acid and 1ml of 0.02N brominating mixture were added. The flasks were shaken well and set aside for 5 min for complete bromination. Then, 1ml of 0.1N potassium iodide was added to each flask and diluted to 25 ml with distilled water. The yellow solution formed was measured at 280nm against distilled water as a blank. Amount of dopamine present in supernatant of rat brain homogenate was read from calibration curve.[15]

Estimation of Catalase:
The assay mixture consisted of 50 µl of 1 M Tris–HCl buffer (pH8.0) containing 5 mM EDTA, 900 µl of 10 mM H2O2, 30 µl of MilliQ (purified/distilled) water and 20 µl of the brain tissue supernatant. The rate of decomposition of hydrogen peroxide was observed spectrophotometrically at 240 nm.[14]

Statistical analysis:
The results of Neuroprotective activity were expressed as Mean ± SEM from 6 animals in each group for rats and 8 zebrafish in each group. Results were statistically analyzed using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and $P<0.05$, $P<0.01$, $P<0.001$ was considered significant.

RESULTS AND DISCUSSION:

The present study was performed to carry out the phytochemical and pharmacological evaluation of ethanolic extract of *Carissa carandas* (EECC). The results are presented in tables and graph format. The result displayed include physicochemical, phytochemical evaluation and confirmatory test for triterpenoids, acute toxicity study for EECC in zebrafish, and neuroprotective activity of EECC and pharmacological effect of EECC on MSG induced excitotoxicity and rotenone induced Parkinson's Disease.

Physicochemical and Preliminary Phytochemical analysis

Table No. 1: Physicochemical parameter evaluation of EECC

Total ash	Acid insoluble ash	Water soluble ash	Loss on drying
13.5%	10.5%	8.5%	4%

Table No. 2: Preliminary Phytochemical analysis

SR. NO	TEST	OBSERVATION	INFERENCE
1	Dragendroff's test (Alkaloids)	Orange colour	Alkaloids may be present
2	Molisch test (Carbohydrates)	Violet ring is formed at the junction of two liquids	Carbohydrates may be present
3	Benedict test (Carbohydrates)	Orange colour	Carbohydrates may be present
4	Lead acetate test (Tannins)	Yellowish white precipitate	Tannins may be present
5	Copper acetate test (Diterpenes)	Green colour	Diterpenes may be present
6	Ferric Chloride test (Phenols)	Red brown colour	Phenols may be absent
7	Alkaline reagent test (Flavonoid)	Yellow colour	Flavonoids may be present
8	Foam test (Saponin)	Formation of foam	Saponin may be present

As per above phytochemical analysis, EECC showed presence of Alkaloids, Carbohydrates, Tannins, Diterpenes, Flavonoids, and Saponin.

Table no 3 : Confirmatory test for triterpenoids (plant origin compound):

SR. NO	CONFIRMATORY TEST	OBSERVATION	INFERENCE
1	Liebermann-Burchard test	Red colour	Triterpenoids was found to be present
2	Salkowski test	Red colour	Triterpenoids was found to be present

Acute toxicity study

In Zebrafish: When Adult zebrafish were exposed to EECC at dose of 100mg/L for period of 96hours no mortality were recorded. Irregularities in movement, catalepsy, freezing episodes were not observed.

Rotenone induced Parkinson's disease in zebrafish

Behavioural parameters observed in zebrafish

Table no. 4 Number of freezing episodes

Time interval (min) / Groups	0	15	30	45	60
	Number of freezing episodes				
Vehicle 10% DMSO	0	0	0	0	0
Rotenone (3pg/ml)	2 ± 0.4225**	1.88 ± 0.29**	1.5 ± 0.19**	1.5 ± 0.18**	1.25 ± 0.41**
Selegiline (0.03 µg/ml)	0	0	0	0	0
EECC (5 µg/ml)	0	0	0	0	0
EECC (10 µg/ml)	0	0	0	0	0

Figure. No. 1 Number of freezing episodes

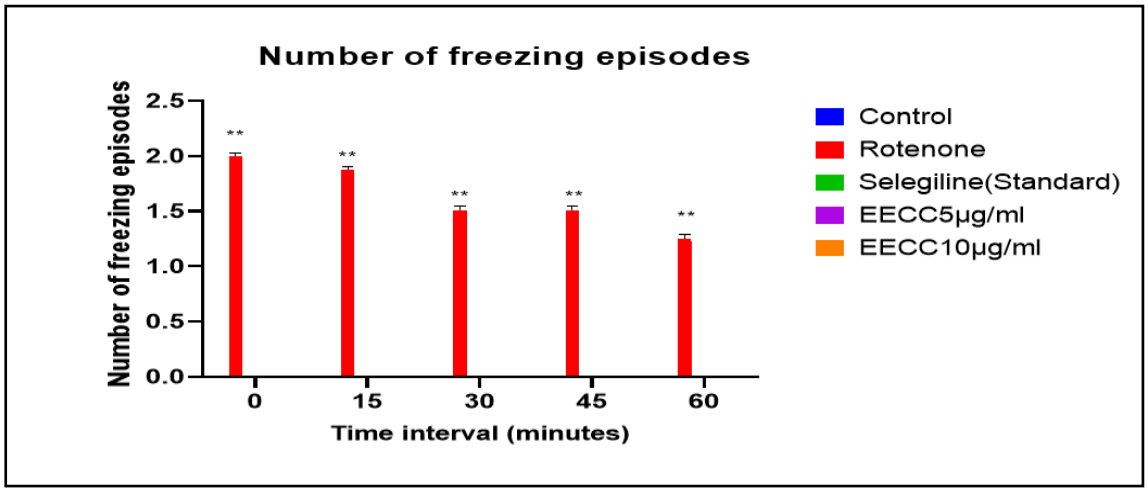


Table. No. 5 Duration of freezing episodes

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	0	0	0	0	0
Rotenone (3pg/ml)	8.16 ± 1.09***	2.38 ± 0.26***	2.12 ± 0.35 ***	2.12 ± 0.22***	1.25 ± 2.56***
Selegiline (0.03 µg/ml)	0	0	0	0	0
EECC (5 µg/ml)	0	0	0	0	0
EECC (10 µg/ml)	0	0	0	0	0

Figure. No. 2 Duration of freezing episodes

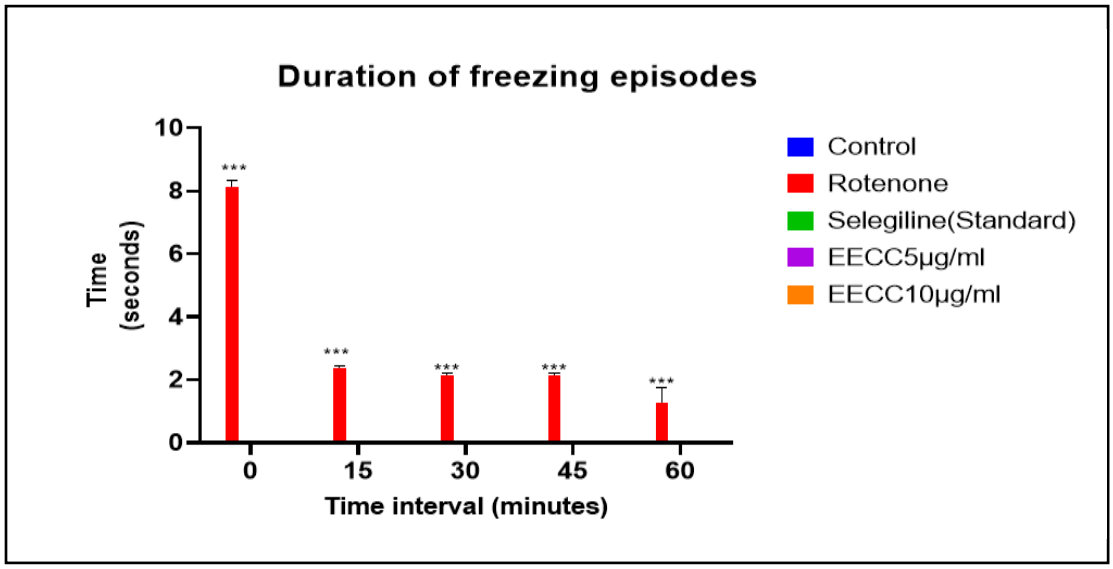


Table. No. 6 Complete cataleptic time

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	0	0	0	0	0
Rotenone (3pg/ml)	13.75 ± 2.39**	9.12 ± 0.47**	8.75 ± 0.72**	7.87 ± 0.71**	4 ± 1.34**
Selegiline (0.03 µg/ml)	0	0	0	0	0
EECC (5 µg/ml)	0	0	0	0	0
EECC (10 µg/ml)	0	0	0	0	0

Figure. No. 3 Complete cataleptic time

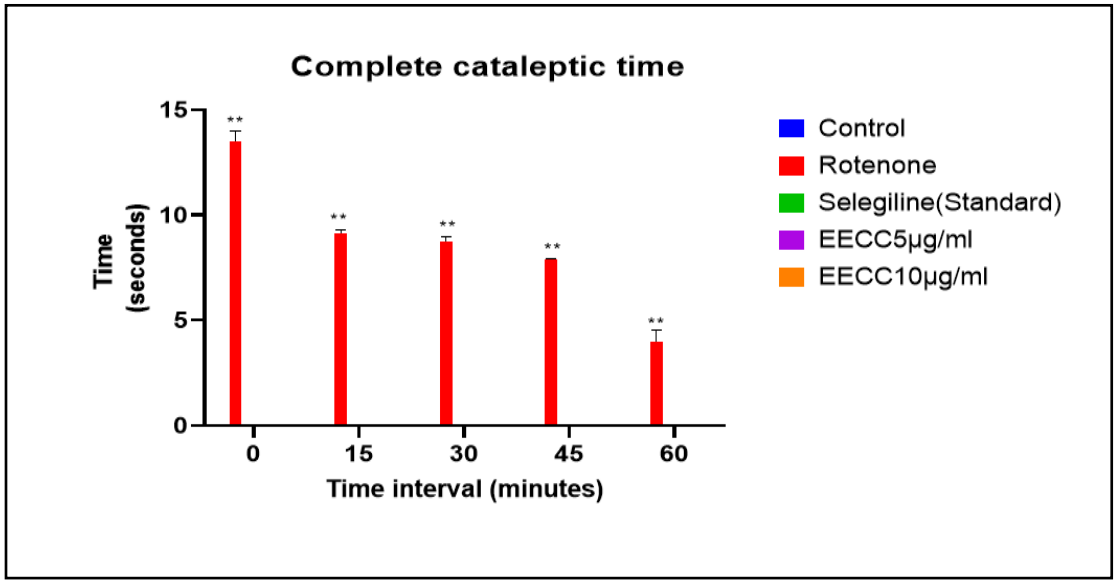


Table. No. 7 Time spent near the bottom of the tank

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	7.35± 1.72	18.5 ± 3.53	20.5 ± 2.45	19.5 ± 2.31	14.88 ± 4.74
Rotenone (3pg/ml)	2.25 ± 1.20*	12 ± 1.70*	13.37± 0.88*	7.5 ± 0.77*	10.62 ± 1.67*
Selegiline (0.03 µg/ml)	5.75 ± 0.55*	14.87 ± 1.368*	15.87 ± 2.27*	16.62 ± 1.87*	11 ± 2.33*
EECC (5 µg/ml)	7.37 ± 2.31**	14.25 ± 2.83**	11.62± 2.90**	13.87 ± 1.51**	8.25 ± 1.64**
EECC (10 µg/ml)	12.37 ± 2.52**	19.87± 3.33**	16.37 ± 2.67**	9.37 ± 1.05**	12.38 ± 3.46**

Figure. No. 4 Time spent near the bottom of the tank

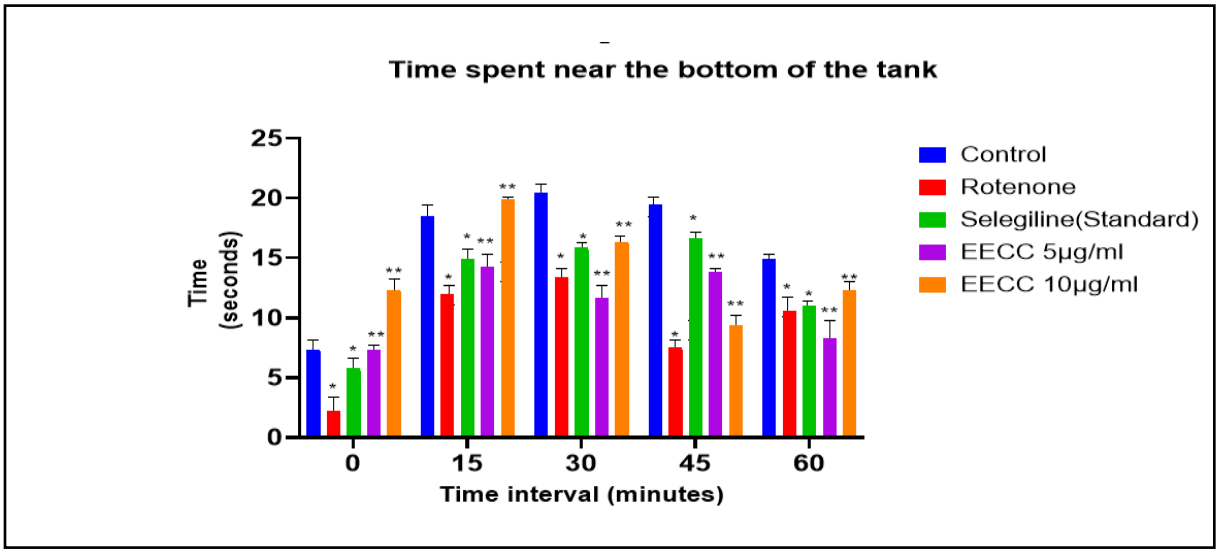


Figure .No. 8 Latency to travel from one point to another

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	6.12± 1.25	4.25± 0.37	8.38 ± 1.49	4.62 ± 0.93	8.25 ± 1.13
Rotenone (3pg/ml)	91.62± 14.16**	10.25 ± 1.48**	8 ± 0.55**	11.25 ± 0.59**	5.75 ± 0.89**
Selegiline (0.03 µg/ml)	7.62± 1.99***	7.12 ± 1.18***	7 ± 0.82***	7.25 ± 1.03***	8.13 ± 1.14***
EECC (5 µg/ml)	8.75± 1.31**	7.87 ± 0.71**	10.12 ± 1.55**	6.12 ± 0.85**	6.62 ± 6.62**
EECC (10 µg/ml)	7.87 ± 0.61**	5.13 ± 0.29**	6.63± 0.96**	5.75 ± 1.14**	4.37 ±0.70**

Figure. No.5 Latency to travel from one point to another

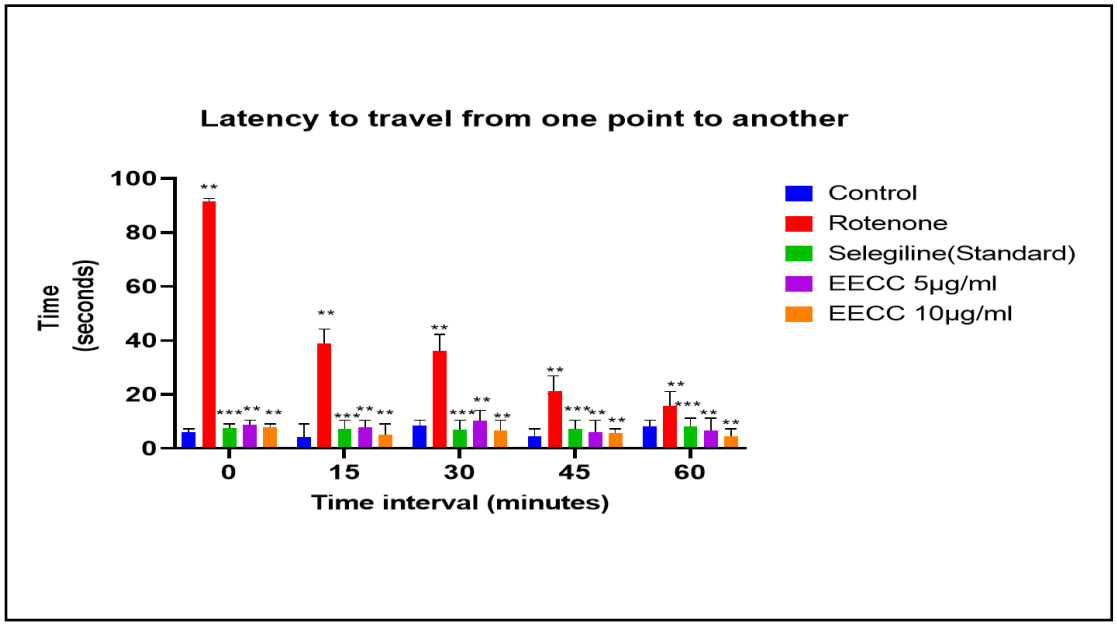


Figure. No. 9 Total distance moved

Time interval (min) / Groups	0	15	30	45	60
	Distance moved in cm				
Vehicle 10% DMSO	549.75 ± 35.89	592.5 ± 70.29	618.75 ± 58.26	622.5 ± 26.14	616.5 ± 23.53
Rotenone (3pg/ml)	70.5 ± 29.23*	312 ± 16.62*	303 ± 23.91*	311.25 ± 9.65*	343.5 ± 15.94*
Selegiline (0.03 µg/ml)	541.75 ± 45.32***	546 ± 23.97***	458.25 ± 21.92***	551.25± 28.03***	624.25 ± 22.01***
EECC (5 µg/ml)	501 ± 43.01***	435 ± 29.39***	420 ± 47.67***	537.75 ± 33.03***	612.60 ± 22.98***
EECC (10 µg/ml)	509.25 ± 22.49***	546.75 ± 64.56***	518.25 ± 19.24***	555.75 ± 23.62***	694.75 ± 19.68***

Figure. No.6 Total distance moved

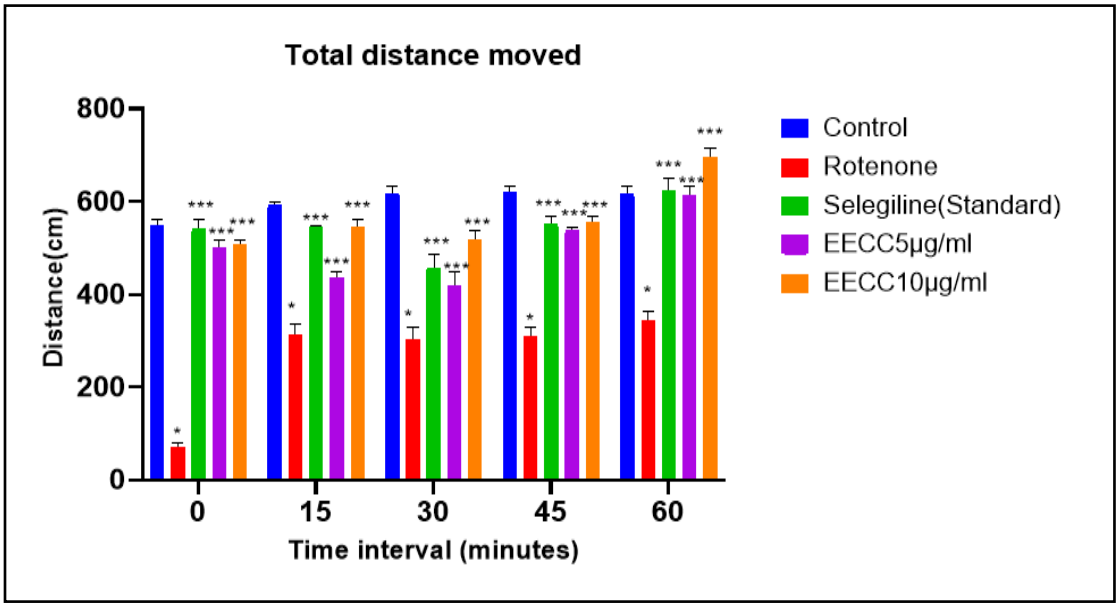
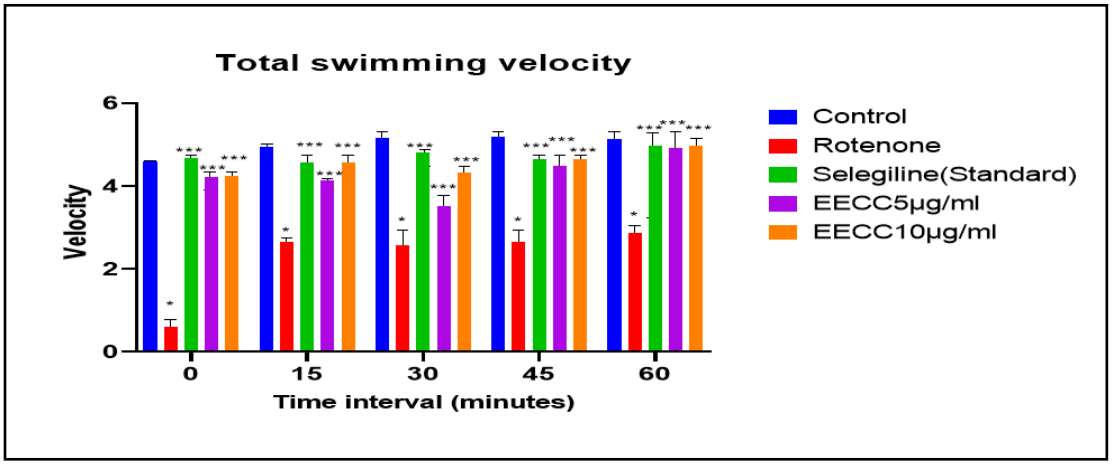


Table. No. 10 Swimming velocity

Time interval (min) / Groups	0	15	30	45	60
	Velocity (cm/s)				
Vehicle 10% DMSO	4.58 ± 0.29	4.9 ± 0.59	5.15 ± 0.48	5.19 ± 0.22	5.13 ± 0.19
Rotenone (3pg/ml)	0.58 ± 0.24*	2.64± 0.13*	2.56 ± 0.20*	2.63 ± 0.07*	2.86 ± 0.13*
Selegiline (0.03 µg/ml)	4.68 ± 0.37***	4.55 ± 0.19***	4.81 ± 0.18***	4.63 ± 0.25***	4.96 ± 0.18***
EECC (5 µg/ml)	4.22 ± 0.41***	4.12 ± 0.66***	3.5 ± 0.39***	4.48 ± 0.27***	5.08 ± 0.18***
EECC (10 µg/ml)	4.24 ± 0.18***	4.55 ± 0.53***	4.31 ± 0.16***	4.63 ± 0.19***	4.96± 0.16***

Figure. No. 7 Swimming velocity



Monosodium glutamate induced excitotoxicity in rats

Behavioural parameters: Were observed on 0, 3rd ,5th ,7th day of main study.

Table .No. 11 Locomotor activity by Actophotometer

Day / Groups	0	3	5	7
	Score of Locomotor activity (Counts/5min)			
Vehicle (1% CMC)	175.5± 8.40	174.5± 11.20	176.16±8.576	184± 14.6
Disease control (MSG 2g/kg)	181.83± 2.00*	107.16±6.63*	97.33±4.24*	76±4.50*
Standard (Selegiline 10mg/kg)	182.33± 14.11**	169.16± 11.69**	161.5±4.12**	157.16 ±3.24**
EECC (200mg/kg)	178.16±14.67**	139.83±19.37**	131.33±13.61**	123.83±11.62**
EECC (400mg/kg)	179.66± 16.86**	152.33± 12.28**	150.83± 12.41**	146.33± 11.06**

Figure. No. 8 Actophotometer

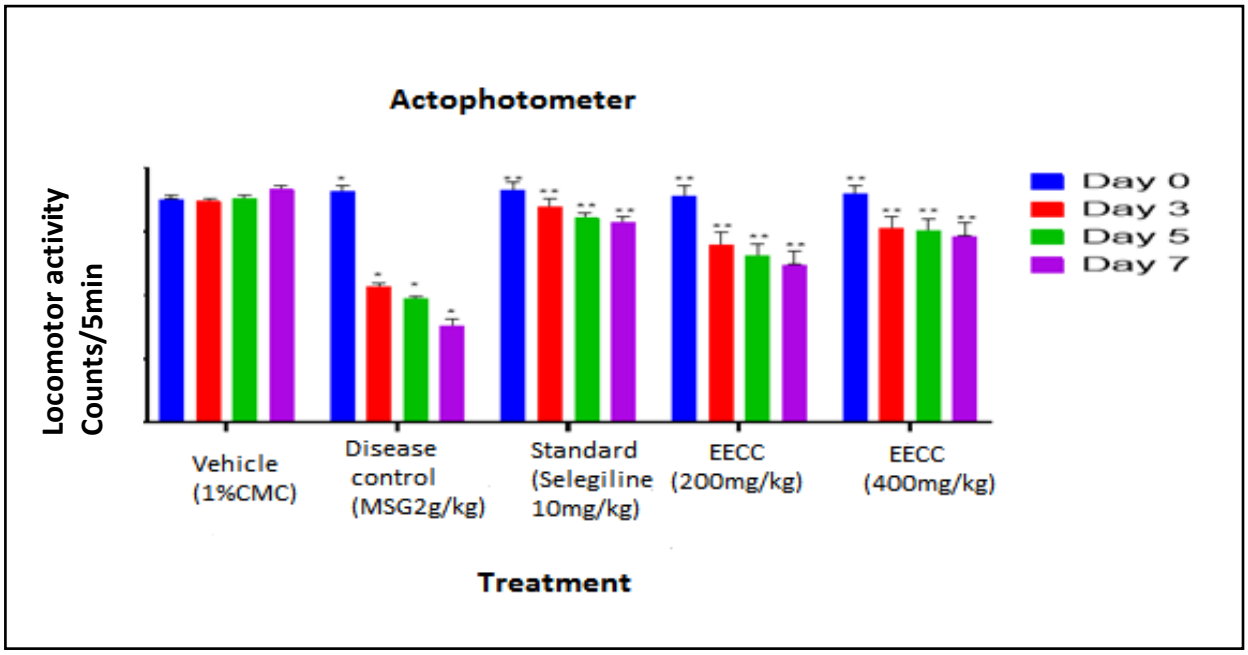


Figure. No. 12 Horizontal bar test

Day / Groups	0	3	5	7
	Fall of time (Sec)			
Vehicle (1% CMC)	19.33± 2.74	19.67± 2.77	18.17±1.58	22± 2.14
Disease control (MSG 2g/kg)	18.33± 2.06*	9.5 ± 1.54*	7.33±1.96*	4.83±0.95*
Standard (Selegiline 10mg/kg)	19.66±2.90**	16.66±1.47**	16.83±1.40**	20.33±1.28**
EECC (200mg/kg)	19.16±1.10**	12.33±2.33**	12.83±1.40**	14.33±1.40**
EECC (400mg/kg)	19.5±2.90**	16.5±2.46**	16.66± 1.20**	17±2.96**

Figure. No .9 Horizontal bar test

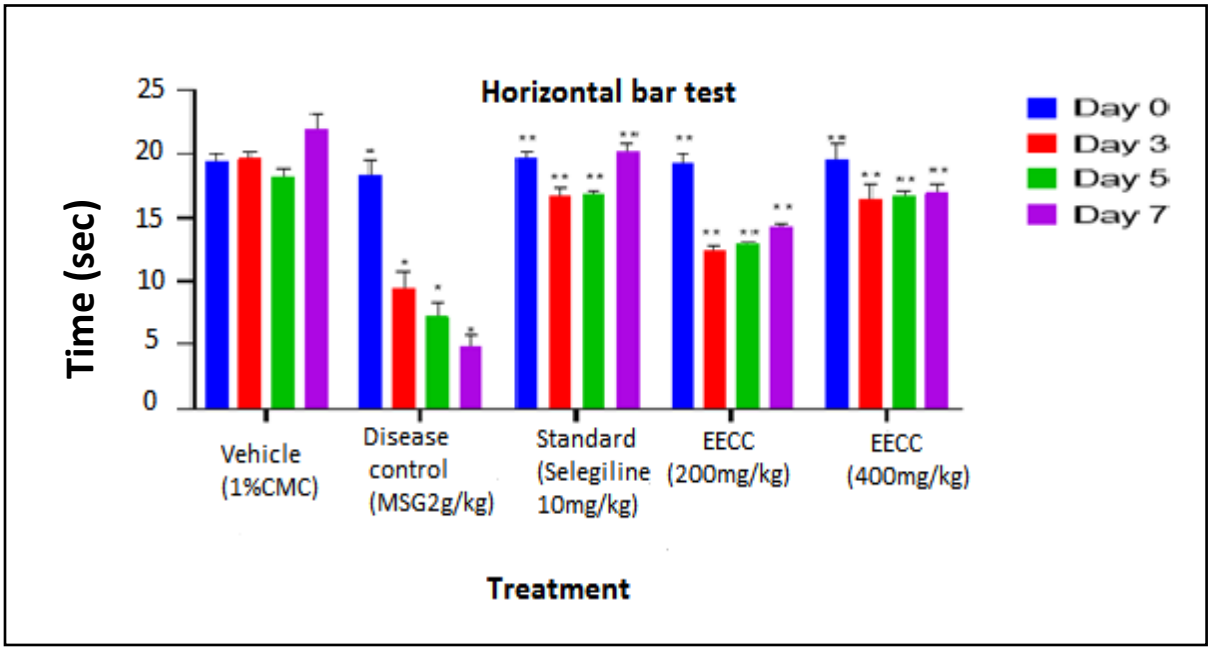


Table. No. 13 Hang test

Day / Groups	0	3	5	7
	Fall of time (Sec)			
Vehicle (1% CMC)	17.83± 3.55	16± 2.33	19± 2.9	21.33± 3.14
Disease control (MSG 2g/kg)	18.17± 2.67*	6± 1.18*	6± 1.07*	5± 0.93*
Standard (Selegiline 10mg/kg)	18.5± 0.99**	15.67± 1.76**	16.83± 2.01**	18.17± 2.83**
EECC (200mg/kg)	17.16± 2.77**	11.66±1.60**	14.33± 3.26**	15.67 ± 2.90**
EECC (400mg/kg)	18± 1.67**	12.5 ± 1.33**	16.33± 2.04**	17.5 ± 2.92**

Figure. No. 10 Hang test

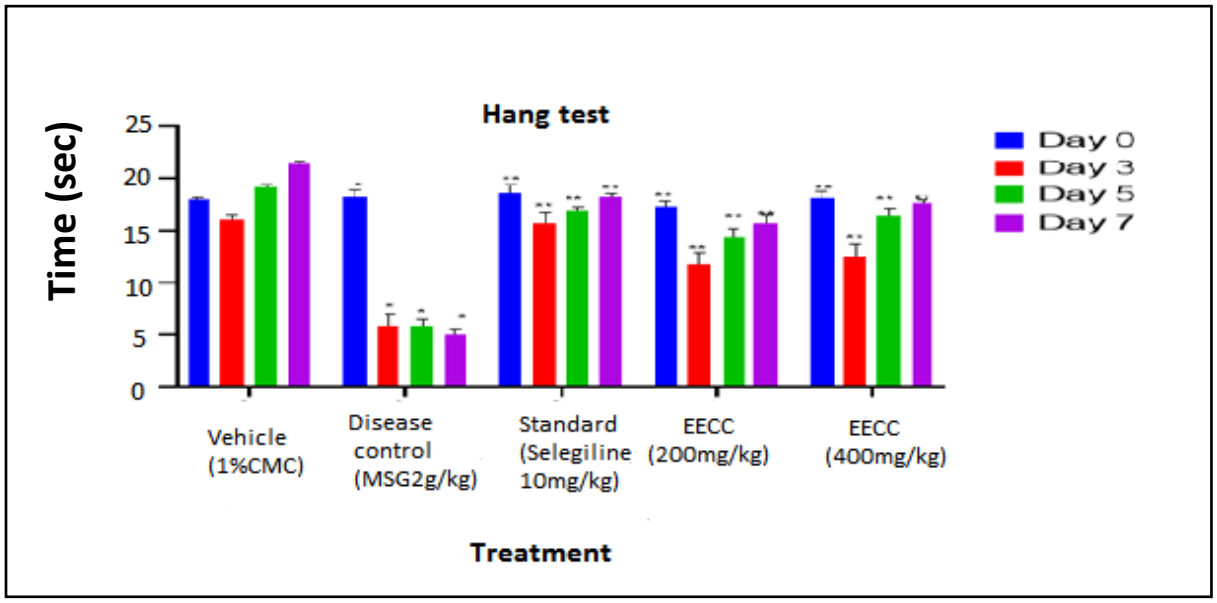


Table.No. 14 Biochemical estimation

Groups	GLUTATHIONE (µg/mg protein)	DOPAMINE (mg/g protein)	CATALASE µmoles/g tissue
Vehicle (1% CMC)	24.85± 0.97	5.72± 0.62	63.37± 0.63
Disease control (MSG 2g/kg)	7.24 ± 0.33**	2.08± 0.70**	26.83± 0.62*
Standard (Selegiline 10mg/kg)	21.212± 0.940***	5.22± 0.01**	59.34± 0.70**
EECC (200mg/kg)	17.65± 0.35**	3.78± 0.03**	48.69± 1.61**
EECC (400mg/kg)	20.93± 0.41**	4.98± 0.05**	57.42± 1.19**

Figure. No. 11 Estimation of Glutathione

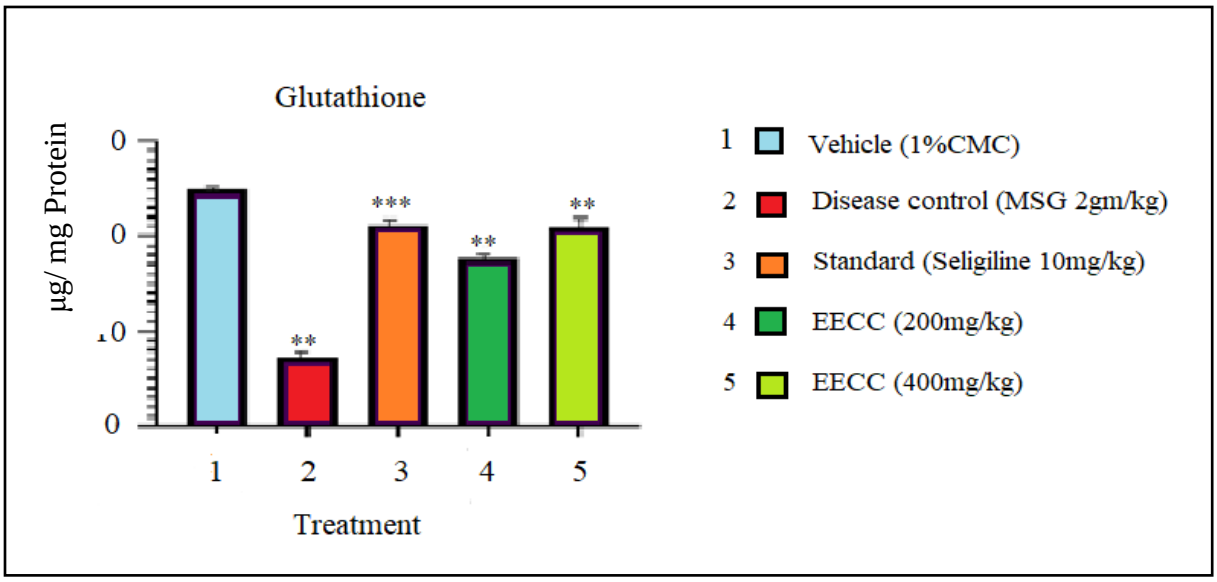
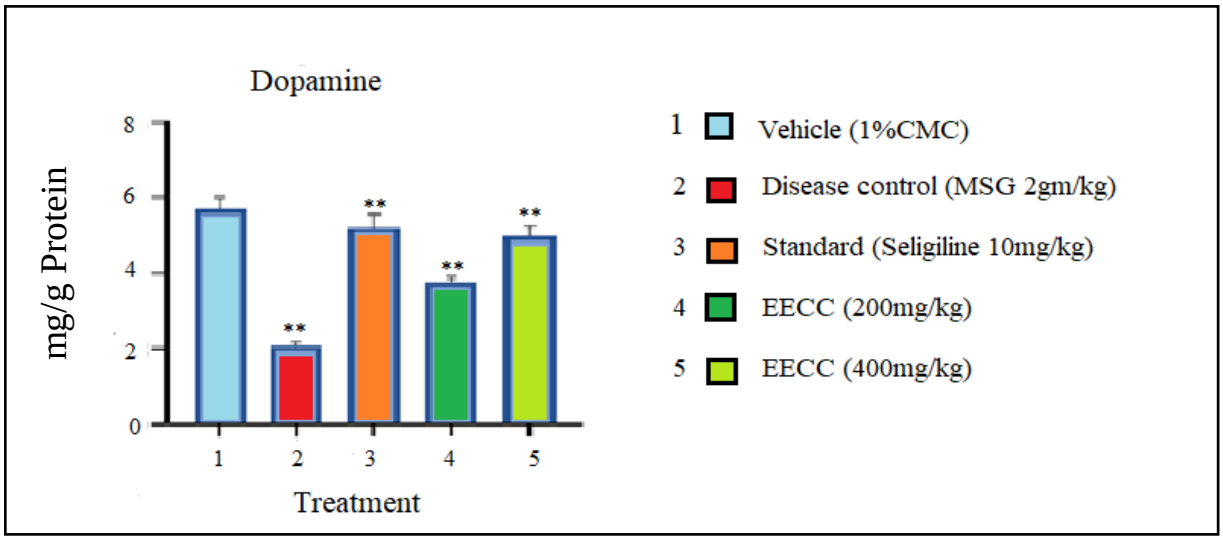
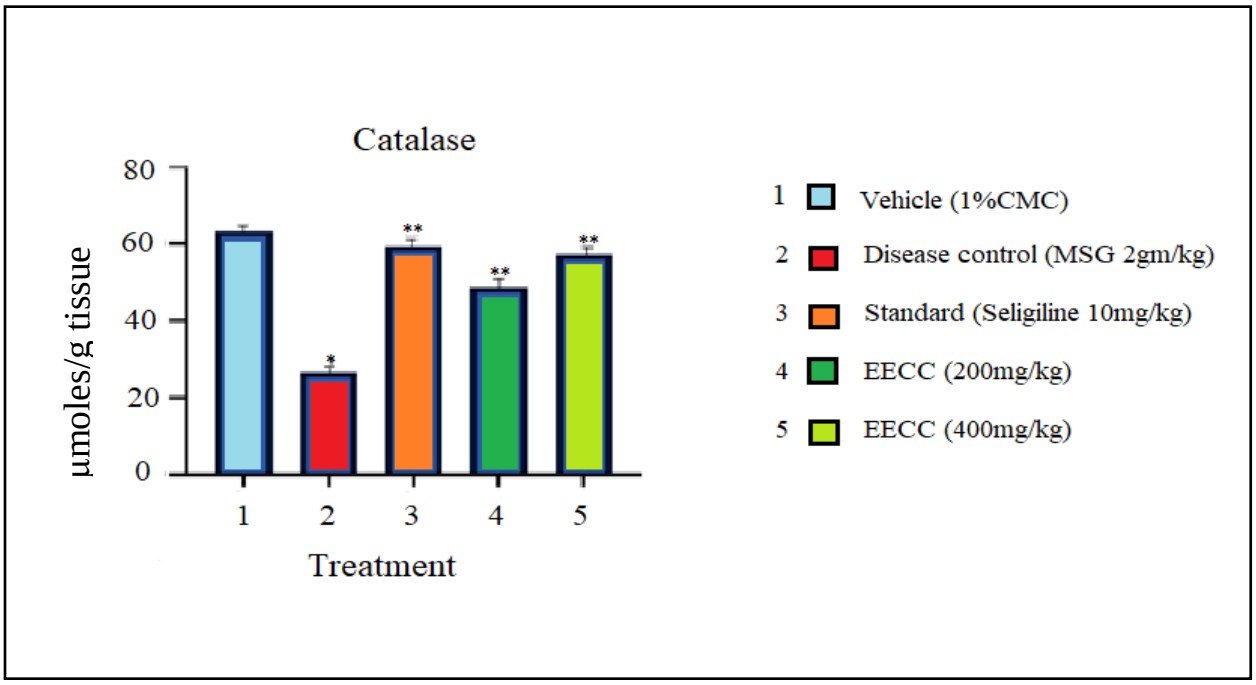


Figure .No. 12 Estimation of Dopamine





DISCUSSION:

Parkinson's disease being incurable, the present pharmacotherapy strategy is based on treating one or more symptoms. With side effects, the synthetic drugs are being replaced by herbal drugs. Over many years herbal plants have overtaken synthetic drugs majorly in treating CNS disorders. With their natural origin and combination of bioactive compounds present in them make them an ideal drug. Considering excitotoxicity as one of the major mechanisms, the herbal plant *Carissa carandas* was selected to evaluate its neuroprotective activity against excitotoxicity model of Parkinson's disease.

Ethanolic extract of Carissa carandas is known to exhibit NMDA antagonism. The probable mechanism is by blocking NMDA receptor, leading to decrease in Ca2+ further causing decrease in depolarization and neuronal excitotoxicity.

Phytochemical screening of EECC showed presence of alkaloid, carbohydrate, saponin, flavonoids, tannin, diterpene which may contribute to its activity. Confirmation of presence of triterpenoids gave us assurance that the plant will show activity against excitotoxicity as they are known to be effective against excitotoxicity.

In this study, neuroprotective activity of *Carissa carandas* was evaluated in lower species- Zebrafish as well as higher species- Rodents (Rats) to get a complete idea of its activity, which helped us to understand its efficacy and dose difference for its activity in different species. Two models i.e rotenone induced Parkinson's disease in zebrafish and MSG induced excitotoxicity in rats was evaluated.

Rotenone a very toxic pesticide was chosen as it indirectly causes excitotoxicity by inhibiting Mitochondrial complex I. Monosodium glutamate another toxin was used to induce Parkinson's disease as it causes overactivation of NMDA receptor and causes excitotoxicity leading to Parkinson's disease. To have a comparative analysis, a Selegiline which acts as NMDA antagonist was used as standard. Acute toxicity in zebrafish was carried out by OECD guidelines 203. EECC did not show any signs of toxicity and mortality rate was 0%. Acute toxicity in rodents were performed earlier hence was not performed.

Rotenone, a neurotoxin when administered in zebrafish, it showed to reproduce behavioural features similar to that of Parkinson's disease. Sign of PD is rigidity of fins, slowness of movement, freezing episodes, catalepsy. Significant decrease in movement, catalepsy along with increase in freezing episodes was observed when zebrafish were exposed to rotenone. Decrease in locomotor activity is correlated to decrease in dopamine neurons which may be associated to neurodegeneration of neurons which

may be due to neuronal toxicity induced by rotenone. This toxicity can be related to the mechanism of excitotoxicity induced by rotenone. When EECC of dose of 5µg/ml and 10µg/ml was exposed, it showed to increase latency to travel from one point to another and total distance moved and swimming velocity in a dose dependent manner. Also, catalepsy and freezing episodes were diminished. According to the observations, EECC showed promising effect in improving Parkinson's disease.

MSG induced excitotoxicity resulted in behaviour alterations in rats and decrease in concentrations of dopamine and antioxidants such as glutathione and catalase present in the brain. The locomotor activity, neuromuscular strength was increased and muscle rigidity was decreased in EECC treated animals when compared to toxic group. MSG also resulted in decrease in Glutathione leading to increase in Ca^{2+} ions and further decreasing Dopamine and catalase. Whereas dopamine, glutathione and catalase concentration were found to be restored in EECC administered group. The increase in GSH and catalase help in decrease in excitotoxicity induced free radical generation. The results of EECC were comparable with Standard- Selegiline, which also increased levels of dopamine, glutathione and catalase along with improving behavioural characteristics of animals.

CONCLUSION:

The present study provides evidence that EECC possesses promising activity in improving all symptoms of PD. Decrease in excitotoxicity and neuronal degeneration was found, which may be due to its capability to antagonize NMDA receptor. Also, presence of triterpenoids must have contributed in its activity. It was observed that Behavioural abnormality in zebrafish and rats induced by rotenone and MSG respectively was improved with administration with EECC.

Hence the result conclusively proves that EECC possesses neuroprotective activity against excitotoxicity model of Parkinson's disease.

Further isolation and standardization of bioactive compound can help in finding new insights in its activity.

Acknowledgement

The author Mrs. Rachana D. Sarawade guided author Pereira Veronica Belchir provided guidance about the contents of the research article. The author Pereira Veronica Belchir obtained the data, analysed the data and compiled the data in proper manner and computed the data as per journals Instructions.

Conflicts of interest

None

Abbreviations

MSG- Monosodium glutamate

NMDA-N-methyl-D-aspartate

EECC- Ethanolic extract of *Carissa carandas*

IAEC-Institutional animal ethics committee

PD- Parkinson;s disease

CPCSEA-Committee for the purpose of control and supervision on animals

REFERENCES:

1. Gitler AD, Dhillon P, Shorter J. Neurodegenerative disease: models, mechanisms, and a new hope.
2. Glutamate, Calcium, NMDA Receptor: Their Contribution to Parkinson's disease vero
3. Hughes RC. Parkinson's Disease and its Management. BMJ: British Medical Journal. 1994 Jan 22;308(6923):281.
4. Rabiei Z, Solati K, Amini-Khoei H. Phytotherapy in treatment of Parkinson's disease: a review. Pharmaceutical biology. 2019 Jan 1;57(1):355-62.
5. Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS. Anticonvulsant activity of Carissa carandas Linn. root extract in experimental mice. Tropical Journal of Pharmaceutical Research. 2009;8(2).
6. Sawant RS, Godghate AG. Comparative studies of phytochemical screening of Carissa carandus Linn. Asian J Plant Sci Res. 2013;3:21-5.
7. Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008 Sep 7.
8. Guideline OE. OECD guideline for testing of chemicals–draft proposal for a new guideline–fish embryo toxicity (FET) test
9. Guideline, O.O. 425: acute oral toxicity—up-and-down procedure. *OECD Guidelines for the Testing of Chemicals*. 2001, 2, 12-16.
10. Hegde K, Joshi AB. Hepatoprotective effect of Carissa carandas Linn root extract against CCl 4 and paracetamol induced hepatic oxidative stress.
11. Makhija DT, Jagtap AG. Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: comparison with rat model. Journal of pharmacology and pharmacotherapeutics. 2014 Jan;5(1):39
12. Swamy AV, Patel NL, Gadad PC, Koti BC, Patel UM, Thippeswamy AH, Manjula DV.

Neuroprotective activity of pongamiapinnata in monosodium glutamate-induced neurotoxicity in rats. Indian Journal of Pharmaceutical Sciences. 2013 Nov;75(6):657.

13. Banu Z, Fatima SJ, Fatima A, Fatima S, Zohra SF, Sultana T. Phytochemical Evaluation and Pharmacological Screening of Antiparkinson's Activity of Allium Sativum In Swiss/Albino Mice. IOSR Journal of Pharmacy. 2016;6(6):1-2.
14. Thonda VS, Kumar SH, Handral M, Sonowal A. Neuroprotective evaluation of ethanolic leaf extract of Dalbergia sissoo in monosodium glutamate induced neurotoxicity in rats. International Journal of Pharmaceutical Sciences and Research (IJPSR). 2014;5(3):829-38.
15. Pawar V, Sarawade R. Neuroprotective Effect of Scoparia dulcis Plant Extract against Parkinson's Model of Excitotoxicity in Rats and Zebrafish Model. International Journal of Pharmacy and Pharmaceutical research.