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Research Article

Behavioural and Neuroprotective Effect of Fish Oil on MPTP Induced Parkinson's Disease in Mice

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ABSTRACT
Parkinson's disease (PD) is a common neurodegenerative disease characterized by progressive and selective loss of dopaminergic neurons in the SNpc that provide innervations in the striatum. The present work was focused on the neuroprotective effect of fish oil (Omega-3 fatty acids) against experimentally (1-Methyl-4-Phenyl-1,2,3,6 tetrahydropyridine) induced Parkinson's disease in mice, by analyzing the behavioural studies. Rota rod test, Hang test, measurement of fore paw stride length during walking test and Photo-actometer tests proved that 5% and 10% fish oil in mice improved the performance against MPTP administration and the results were significant ($p<0.05$). Compared to the control group 5% fish oil treated group shows improved behavioural activity - Rota rod test 71%; Hang test 70%, measurement of fore paw stride length during walking test 34% and Photo-actometer tests 14%. The 10% fish oil treated group shows Rota rod test 86%; Hang test 87%, measurement of fore paw stride length during walking test 64% and Photo-actometer tests 18.45%. The study evidence that fish oil is a neuroprotective against MPTP induced in mice.

Key words: Neuroprotective, Parkinson's disease, MPTP, fish oil, Oxidative stress.

INTRODUCTION

Neurodegenerative diseases represent group of neurological disorders with heterogeneous clinical and pathological expressions affecting specific sites of neurons in specific functional anatomical systems; they arise for unknown reasons and progress in a relentless manner [(1)]. **Parkinson's disease** is degenerative disorder of the central nervous system and main symptoms are tremor at rest, stiffness, slowing of movement and postural instability. Parkinsonian syndromes can be divided into four subtypes according to their origin: primary or idiopathic, secondary or acquired, hereditary parkinsonism, and parkinson plus syndromes or multiple system degeneration. The exact cause of this disease still remains a mystery this hampers the development of proper therapeutic interventions. Despite many approaches and efforts, still now no researchers have successfully developed to cure or modality to check the disease, and most of the therapies only provide functional relief. The neuropathology of the disease is based on depigmentation and cell loss in the dopaminergic nigrostraital tract of the brain, with the corresponding decrease in the striatal dopamine (DA) concentration. Evidence suggests that immense oxidative stress, free radical formation, genetic susceptibility programmed cell death, and another unknown factor like endogenous (or) exogenous.

Oxidative stress appears to play an important role in the sporadic forms of Parkinson's disease and endogenous sources of oxidative stress include the free radicals produced by metabolism of dopamine and melanin. Reactive oxygen species (ROS) are produced during several intracellular pathways, and they induce oxidative stress. Accumulation of ROS can cause oxidative alteration on cell constituents, and the alteration cause an irreversible oxidative damage over lifetime of a cell. Oxidative stress is generally related to generation of several diseases such as Alzheimer's diseases, Parkinson's disease, rheumatoid arthritis, the pathologies caused by diabetes, neuro degeneration in motor neurone disease, and cardiovascular disease[(2)]. Antioxidants were used to represent the chemicals that prevented the consumption of oxygen. For this reason, antioxidants become necessary for the use as supplements to human health. Although several strong synthetic antioxidants have already been reported and used, the antioxidants from natural sources are used as supplements to human health. A wide range of natural compounds including phenolic compounds, nitrogen compounds, and careteniods has good antioxidative properties. The beneficial effects have been attributed to the active ingredients of fish oil, Eicosapentaenoic acid and Docosahexaenoic acid. Hence present study was done to unearth the neuroprotective effect of fish oil (Omega-3fatty acids) in animal model of PD.

Drugs and Chemicals: 1-Methyl -4- Phenyl – 1, 2, 3, 6 tetrahydropyridine Hydrochloride (MPTP HCl) was purchased from M/S Sigma Chemicals Co., St. Louis,

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Fig: 1 Effect of Fish oil on motor coordination in MPTP treated mice assessed by Rotarod test

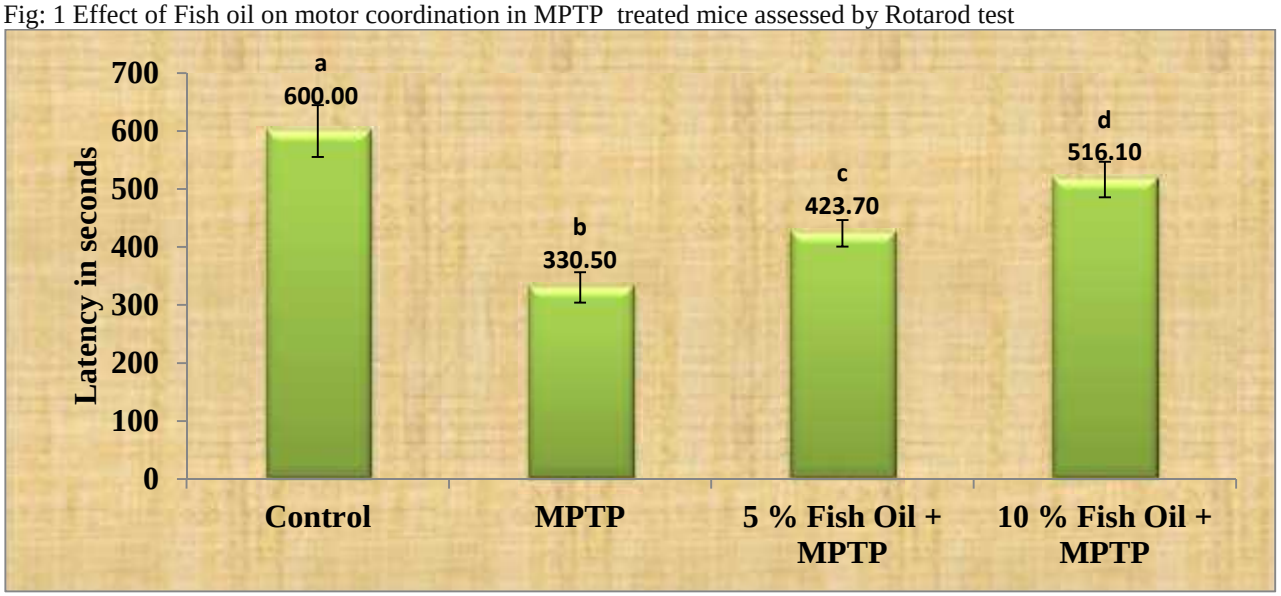


Fig: 2 Effect of Fish oil on neuromuscular strength in MPTP treated mice assessed by Hang test

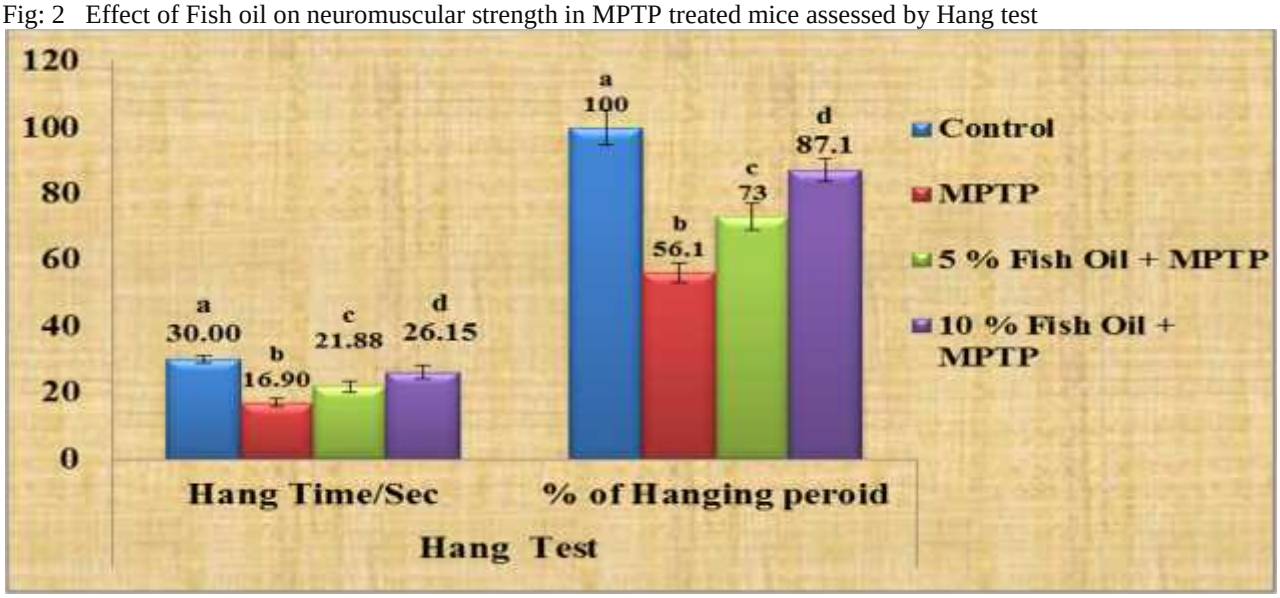


Table: 1 Results of Fish oil on MPTP induced behavioral deficit in mice

Groups	Rotarod test (Latency in seconds)	Hang test (Hang time in Secs)	Hang test (% of Hanging Period)	Photo-actometer (No. of counts/ 60 min)	Fore paw stride length on left side
Control	600.00 ± 44.55[a]	30.00 ± 1.23[a]	100.00 ± 5.16[a]	3810.00 ± 166.67[a]	7.10 ± 0.46[a]
MPTP	330.50± 26.21[b]	16.90± 1.32[b]	56.10 ± 2.84[b]	2144.00 ± 206.77[b]	5.70 ± 0.30[b]
5 % Fish Oil + MPTP	423.70 ± 22.76[c]	21.88 ± 1.73[c]	73.00± 4.19[c]	2526.00± 238.70[c]	6.10 ± 0.42[c]
10%Fish Oil + MPTP	516.10 ± 30.65[d]	26.15 ± 2.03[d]	87.10 ± 3.55[d]	3161.00 ± 316.34[d]	6.50 ± 0.32[d]

Values are expressed as means ï S.D. for eight mice in each group. Values not sharing a common superscript differ significantly at p<0.05 (DMRT)

U.S.A. Soft gelatin capsules (Maxepa) of fish oil was purchased from E-Merk pharmaceuticals, Mumbai, India; and other solvents/reagents were of analytical grade.

Animals: The study was undertaken at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, in accordance with the National Institute of Health "Guide for the care and use of Laboratory Animals" (NIH, 1985). The study was approved by the Animal Ethical Committee of Rajah Muthiah Medical College and Hospital [Registration No 160/1999/ (CPCSIA)] Annamalai Nagar, Tamil Nadu, India [Proposal No.723, dated 19.04.2010]. C57BL/6J black male mice of 6 weeks old were used for this study as they are genetically MPTP sensitive [3]. Animals were

Fig: 3 Effect of Fish oil on spontaneous motor activity in MPTP treated mice assessed by Photo-actometer

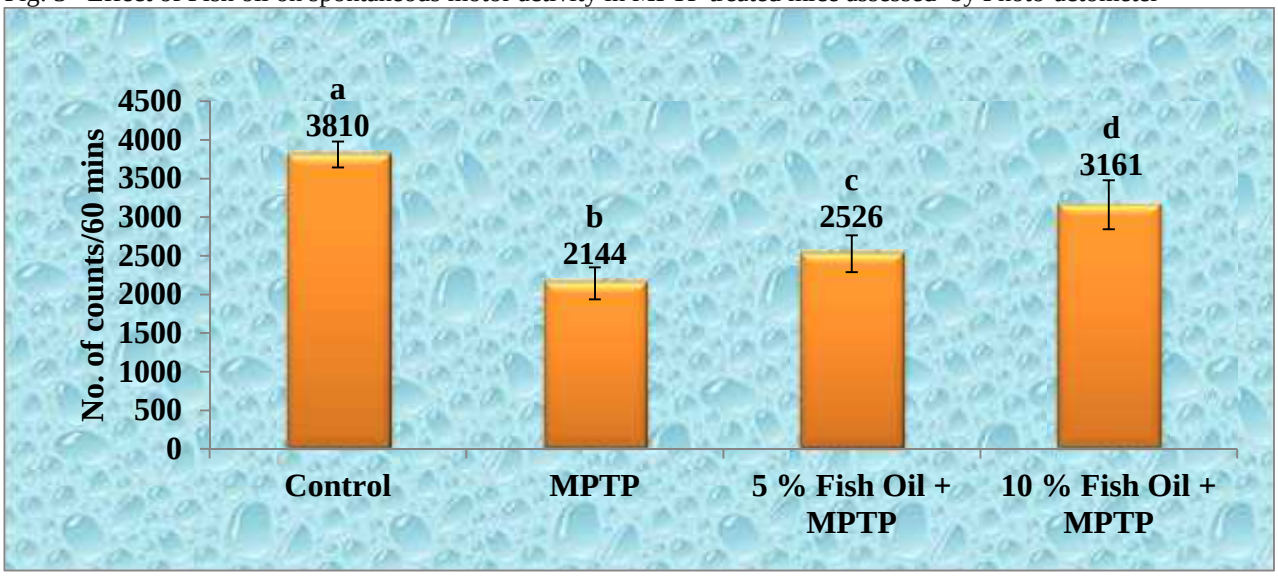
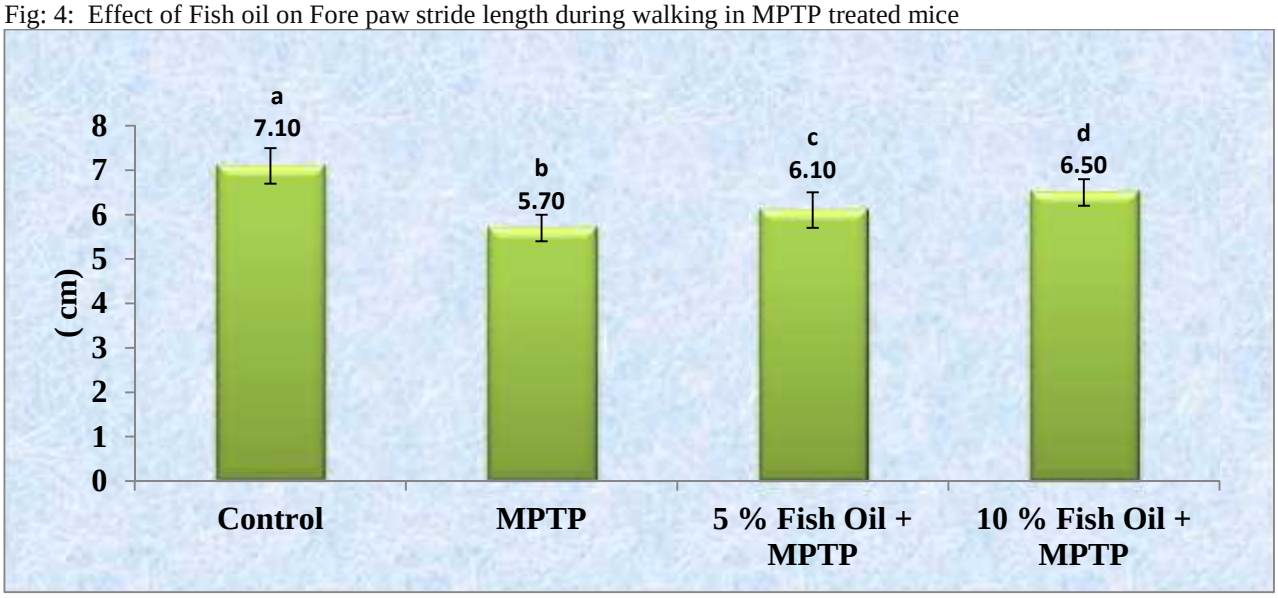


Fig: 4: Effect of Fish oil on Fore paw stride length during walking in MPTP treated mice



housed in well ventilated room (temperature 23±2ºC, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum***.** As per the standard practice, the mice were acclimated 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygiene environment in the animal house. Experimental protocol: C57BL/6J black male mice were randomly divided into four groups of eight each. The animals were housed in the animal house for 40 days. Group-I (normal control) treated with normal diet only, Group-II (experimental control) treated with normal diet only, Group-III fish oil 5% (v/w) mixed with normal diet. Group-IV fish oil 10% (v/w) mixed with normal diet. Fish oil was mixed with standard pellet diet of groups III and IV for 30 days prior to induction of PD. Group I and II received the normal diet only for 30 days. At the end of 30 days, MPTP (10mg/kg) was dissolved in saline, administered I.P, twice at 16 hours interval to groups II, III, and IV to induce PD[4]. After induction of PD, groups III and IV were treated with fish oil for 7 more days while Groups I & II received normal diet.

Behavioural studies

Rota rod test [5, 6] : Motor coordination was assessed using an automated rotarod [MKM-Chennai-India]. Mice were trained for 2 consecutive days prior to MPTP dosing in an acceleration mode (2-13 rpm) for over 2min. The training was repeated at a fixed speed (13 rpm) until the mice were able to stay on the rod for 600 sec. On the 7th day after the last MPTP dosing, the mice were assessed for their coordination capability on the rod at 13 rpm for a maximum recording time of 600 sec. The Rota rod test was performed twice every 30 min and the results were averaged to obtain a single value for each mouse.

Hang test [7] : Neuromuscular strength was determined in the grid hang test; mice were lifted by their tail and slowly

Fig: 5 Fore paw stride length during walking

placed on a horizontal grid (grid 12cm^2 with opening 0.5 cm^2) and supported until they grabbed the gird with both their fore and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was mounted 20cm above a hard surface, to discourage falling but not leading to injury in case of animal fall. The apparatus was equipped with a 3-inch wall to prevent animals from transversing to the upper side of the grid. Animals were required to stay on the grid for 30 seconds. All animals met this criterion before MPTP administration. On the 7th day after the last MPTP dosing, the animals were tested in the grid hang test for 30 sec and 10 chances were given with 1min interval and maximum hanging time was recorded.

The calculation was made as percentage of hanging time

$$= \frac{\text{Maximum hanging time}}{30\ Sec} X\ 100$$

Measurement of fore paw stride length during walking [(6)]: A runway (4.5 cm wide, 50 cm long with borders of 12cm height) was arranged to lead out into a wooden box (20x17cm). The animal's fore paws were wetted with green ink and were made to trot on a strip of paper (4.5cm wide, 48cm long) down the brightly lit runway towards the goal bore. Stride length was determined by measuring manually the distance, between each step on the same side of the body, from the middle toe of the first step to the heel of the second step. The three longest stride lengths (corresponding to maximal velocity) were measured from each run. Paw prints made at the beginning (7cm) and the end (7cm) of the run were excluded because of velocity changes. On the 7th day after the last MPTP dosing, each animal was made to run on the runway. An average of at least four clear steps was calculated. Runs in which the mice made stops (or) obvious decelerations observed by the experimenter were excluded from analysis.

Photo-actometer [(6)] : Spontaneous motor activity was assessed by Photo-actometer (Inco-Ambala, India). Photo-actometer operates on photo electric cells which are connected in circuit with a counter. When the beam of light falling on the photo cell is cut off by the animal, a count is recorded. On the 7th day after the last MPTP dosing, each mouse was placed in the Photo-actometer cage and the locomotor activity in each 10 min period (at 1 minute interval) was measured for 60 min.

RESULTS AND DISCUSSION

Parkinson's disease (PD) is a common neurodegenerative disease characterized by progressive and selective loss of dopaminergic neurons in the SNpc that provide innervations in the striatum. In the present study C57BL/6J mice were used and were observed that 7 days after the last dose of MPTP administration, the mice spent significantly less time on the rotating rod **(Fig: 1)** indicating a loss of motor coordination. Pretreatment with both 5% and 10% fish oil in mice significantly ($P<0.05$) improved the rotarod performance. The neuromuscular strength assessed by Hang test **(Fig: 2)** was found to be significantly decreased in MPTP treated mice and the period of Hanging was only 56% of the control group. Pretreatment with 5% and 10% fish oil enhanced the neuromuscular strength significantly ($P<0.05$) and their hanging periods were 73% and 87% of the control group respectively.

Administration of MPTP also significantly reduced spontaneous locomotor activity (Fig: 3) in mice on the 7th day. Pretreatment with 5% and 10% fish oil significantly ($P<0.05$) attenuated MPTP induced hypo locomotion. The mean stride length of fore limbs in MPTP treated group showed a significant decrease when compared to control group. Pre treatment with 5% and 10% fish oil had significantly ($P<0.05$) increased the fore paw (Fig:4& 5) stride length. Chronic treatment with fish oil decreased the conversion of MPTP into MPP + and also reduced MAO-B activity in the brain of rodents [(8)]. Oxidative stress and mitochondrial dysfunction caused by loss of complex- I activity are identified as some of the primary events leading to neurodegeneration in PD [(9)]. More over fish oil in the present study showed marked anti oxidant activity and this could have protected the dopaminergic neurons from damage by MPTP and thereby improvement in motor dysfunction. Hence present study offer conclusive evidence that fish oil is a neuroprotective. Diet supplemented with deep sea fatty fish or fish oil can protect against neurodegenerative disorders and prevent the progression of neurodegeneration.

REFERENCE

1. Gunduluru Swathi, Gopal reddy, Gari Visweswari and Wudayagiri Rajendra. . Evaluation of rotenone induced parkinson's disease on glutamate metabolism and protective strategies of *Bacopa monnieri. International Journal of Plant, Animal and Environmental Sciences, 62-67.2003.*
2. Rasheed Ahemad.S, Venkataraman S, K.N.Jayaveera, Fazil Ahmed Mohammed and Ibrahim Mohammed. Yashoda Krishna. Effect of hydro-alcoholic root extract of *Nardostachys jatamansi* on haloperidol induced Parkinsonism in Wistar Rats. Pharmacologyonline 2: 1145-1154 (2009).
3. Hamre K, Tharp R, Poon K. Differential strain susceptibility following 1-methyl-4-phenyl

-1,2,3,6- tetra hydropyridine (MPTP) administration acts in an autosomal dominant fashion: quantitative analysis in seven strains of *Musmusculus. Brain Res,* (1999; 828 (1-2):91-103.

4. Mohanakumar KP, Haobam R, Sindhu KM. Swim-test as a function motor impairment in MPTP model of Parkinson's disease: A comparative study in two mouse strains. *Behavioural Brain Research. 2005*. 163:159-167.
5. Sedelis M, Schwarting RK, Huston JP. Behavioral phenol typing of the MPTP mouse model of Parkinson's disease. *Behav Brain Res* .2001, 125:109-125.
6. Tillerson JL, Michael Caudle W, Reverson ME. Detection of behavioural impairments correlated to neuro chemical deficits in mice treated with moderate doses of 1-methyi-4-phenyl-1,2,3,6-tetrahydropyridine. *Exp Neuro* 2002, 178: 80- 90.
7. Tillerson JL, Miller GW. Grid performance test to measure behavioural impairment in the MPTP-treated mouse model of Parkinsonism. *J Neurosci Meth*. 2003, 123:189-200.
8. Chalon S, Delion-Vancassel S, Belzung C. Dietary fish oil affects monoaminergic neurotransmission and behaviour in rats. *J Nutr*. 1998, 128:2512–2519.
9. Delion S, Chalon S, Guilloteau D. Age-related changes in phospholipid fatty acid composition and monoaminergic neuro- transmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty acid-deficient diet. *J Lipid Res* . 1997, *38:680* – 689