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Evaluation of efficiency omega 3 fatty acid improves the behavioural phenotype and protects against oxidative stress against MPP^+ induces Parkinson's disease in mice

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Abstract: This experiment proposed to study the efficiency omega 3 fatty acid on behavioural phenotype of Parkinson's disease (PD) in mice. Totally 7 groups (each group 6 mice) were used in this assessment, each groups were treated with saline (control), MPP^+, L-DOPA, Omega 3 oil, Omega 3 oil (three different concentrations) $+MPP^+$ separately. The behavioral assessments such as bar test, open field test, maze test, hang test were noted on 7th, 14th, 21st and 28th day. After the examination period, the tested animals' midbrains and frontal cortex were dissected to analyze TBARS, GSH, Catalase, Superoxide Dismutase and Glutathione Peroxidase assay. In the bar test, 500mg omega 3 fatty acid administrated mice showed a high cataleptic scores. In open field Test, significant reductions in behavior analysis were observed from the tested mice group. Maze test and hang test doesn't show much difference. In biochemical test, tested groups showed promising results compared to control group. The result strongly proved that the omega 3 fatty acid has remarkable abilities to control the neurodegenerative diseases.

Keywords: Omega 3 oil, Parkinson's disease, Hang test, Maze test, open field test and catalepsy test.

INTRODUCTION

Parkinson's disease is the second most widespread age related neurodegenerative disorder which affects people aged over 55years. This neurodegenerative disease means amyloid development of a human protein called alpha-synuclein and also called as a 'Parkinson's protein'. Major behavioral symptoms of PD are bradykinesia, inactive shake, inflexibility, postural instability and "masked" facial expression Zhao *et al.,* (2010). PD is basically related on the progressive degradation of dopaminergic neurons of the nigro striatal path in the brain. Damage of these neurons shows a reduction in striatal dopamine [DA] substance.

The study of the disease proves that 95% of patients experienced PD with certain symptom like effects of a sporadic type of the disorder and other 5% of PD cases are because of familial bunches. A mixture of hazard factors have been recorded for sporadic PD, including introduction to pesticides and different toxins, positive family ancestry and oophorectomy, among them age remains most significant until now (Veenvliet and Smid, 2014). Therefore vital investigations and novel treatments are needed to maintain the neurons from the degradation and improve the behavior of mind ischemia and neurodegenerative disorder (Feger and Hirsch, 2015).

Now, new findings exposed the benefits of fish consumption for preventing Parkinson's disease. A protein known as parvalbumin, which is found in few distinctive fish variety seems to help forestall the development of certain protein composition firmly connected with Parkinson's disorders. Omega-3 fatty acids have an essential role in optimum brain function by facilitating fluidity in neuronal membranes and regulating of neurotransmitters (Yehuda *et al.,* 1999). DHA supplementation has been found to reconstruct memory and intellectual damages and more seasoned grown-ups with age related psychological decrease without symptoms (Mauro *et al.,* 2010). It has also been discovered that omega-3 supplementation may upgrade protection from free radical attack and lessen lipid peroxidation and might be a powerful dietary enhancement in the administration of different ailments in which oxidant/cell reinforcement balance is upset as in matured cerebrum tissue (Avramovic *et al.,* 2012). Considering the potential modulatory impact of omega-3oil supplementation on PD progress, this examination intended to assess efficiency omega 3 oil improves the behavioural phenotype and protect against oxidative stress in MPP^+ induced Parkinson's disease in mice.

MATERIALS AND METHODS

Animals

In this assessment, Male Swiss albino mice (20-25g) were purchased from the Central Animal House of the Institution. The mice were kept up in detached huge extensive, sterile enclosures throughout the test time frame. It were held under 12hrs light/dim cycles, at 22ºC and 40-60% humidity with nourishment and water. All examinations were completed by the accompanying understanding with the National Institutes of Health

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Guide for the Care and Use of Laboratory Animals (USA), approved by the local ethics committee Reg. No: (173/2007/CPCSEA/19.05.2017) and fit in with guidelines indicated by the animal care and use.

Chemicals and Reagents

1-methyl-4-phenylpyridinium, (MPP^+), OMEGA-3 oil were obtained from Sigma aldrich Chemical Co. Every single chemicals utilized were of expository evaluation. Stock solutions of all chemicals were set up in double distilled water and the dilutions were made new upon the onset of the trial.

Experimental Protocol

The mice were randomized and partitioned into six groups (n=6). All the trials were carried for 28 days

Group I: Saline (10mL/kg (*i.p*)), served as control.

Group II: 3µl MPP^+ (20mg/kg) (*i.p*)

Group III: L-DOPA (Standard) (6 mg/kg orally)

Group IV: OMEGA-3 oil (300mg/kg orally)

Group V: OMEGA-3 oil daily (200mg/kg orally) 1 hrs before 3µl MPP^+ (20mg/kg (*i.p*))

Group VI: OMEGA-3 oil daily (300mg/kg orally) before 3µl MPP^+ (20mg/kg (*i.p*))

Group VII: omega-3 oil daily (500mg/kg orally) before 3µl MPP^+ (20mg/kg (*i.p*))

One hour after the 7^{th}, 14^{th}, 21^{th} and 28^{th} daily administration of MPP^+ or test solution all the mice were tested for behavioural assessment such as Bar Test, Open Field Test, Maze Test, Hang Test.

Catalepsy Test (Bar Test)

Catalepsy test was executed according to the modified method of Kanchana *et al.,* (2018). This process was rehashed 5 times, taking the mean of the 3 longest accounts as catalepsy score.

Open Field Test

The open field test was performed according to the descriptions of Su *et al.,* (2018). The entire investigation was preceded as visually impaired examination.

Maze Test

Maze test was followed the method of Campos *et al*., (2013). Trained mice were used in this test. The food was the reward for finding the way. The animals were put consistently in a similar spot of the Maze (start place), just a single animal at once. At regular intervals the rodents were feed without impediments (after test) for 30 min. Mouse behavior was observed for 30 minutes.

Hang Test

Hang test was conducted following the method of Wrangel *et al*., (2015). Mice were permitted to remain on the grid for 30 sec and 10 possibilities were specified with 5 min interim and the best drop esteems were noted.

Biochemical analysis

Dissection and Homogenization

The mice were decapitated on 29^{th} day after behavioural assessment. The frontal cortex and midbrains were dissected out. Homogenate (10% tissue in 0.1M phosphate buffer (pH 7.4)) were prepared and centrifuged at 10,000×g for 20 min and supernatant was used for biochemical investigation.

TBARS assay

TBARS was examined according to the procedure depicted by Gateva *et al*. (2020).

GSH Assay

Decreased glutathione were examined by the process of Wei *et al*. (2019).

Catalase assay

Catalase assay was examined according to the process describe by Chen *et al.* (2015).

Superoxide Dismutase assay

Superoxide dismutase assay was examined according to the procedure depicted by Wang *et al.* (2011).

Glutathione Peroxidase assay

Determination of glutathione peroxidase (GPx) activity was examined according to the procedure described by Wang *et al*. (2011).

Sample Preparation for Western Blotting

The mid brain cells was harvested and centrifuged at 20 000 rpm for 7 min. The supernatant was blend with 2 × SDS-PAGE test buffer. Brain cells were lysed by RIPA buffer supplemented with protease inhibitor cocktail and centrifuged at 20,000 rpm for 5 min and samples for SDS-PAGE were boiled at 95°C for 1 min.

Western Blotting

SDS-PAGE and immune-blotting were preceded as depicted by Nam *et al.* (2018).

Data Analysis

All data analytics were showing in Mean ± SD in no. of tests (n=6). Statistical outcome was assessed by one-way analysis of variance (ANOVA) using SPSS version 17 software and individual correlations were acquired and utilizing Duncan's Multiple Range Test (DMRT). Data were measured statistically significant.

RESULTS

As a result, Catalepsy test (fig. 1) explored the cataleptic scores increased in the test group's and showed significant difference according to the dose dependent ($p<0.05$). The MPP^+ treated group (Group II) showed low cataleptic score compared to treated mice (Groups V, VI

and VII). In the test groups, high dose level (Group VII) showed high cataleptic scores in the 28th day compared to the other treatments days. This experiment proves that high dose of omega 3 oil can alter the behavior of the animals.

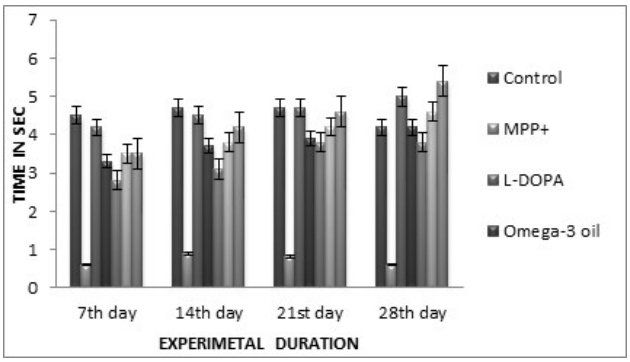

Fig. 1: Effects of Catalepsy test of drug treated mice

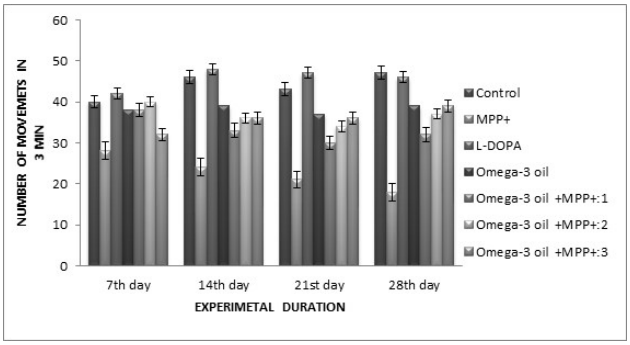

Fig. 2: Shows the peripheral movements of drug treated mice

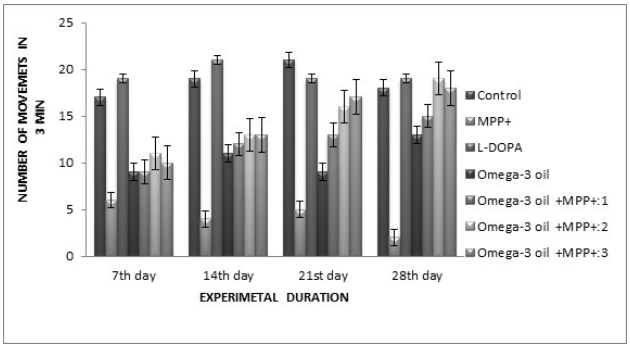

Fig. 3: Shows the central movements of drug treated mice

Open field Test (fig. 2, fig. 3, fig. 4 and fig. 5) showed the significant reduction in behavior analysis such as peripheral movements, rearing, grooming and central movements in tested group mice (group V,VI and VII) compare to MPP^+ injected group mice (group II). Pretreatment of omega 3 oil on mice (group V, VI and VII) made them to express notable improvement in peripheral and central activities along with rearing and grooming in 14th 21th and 28th day treatment ($p<0.05$).

Maze test (fig. 6) showed a major difference on food finding time in experimental and control groups. The food finding time increased in MPP^+ injected mice while comparison with control mice. The time in which the mice found the food at the end of the maze was significantly reduced after omega 3 oil pretreated group (14th, 21nd and 28th day) in comparison with MPP^+ group. There is no much significant changes in omega 3 oil (group V, VI and VII) alone treated mice compared to control animals. In Hang test (fig. 7) hang time expressed the significant decrease ($p<0.05$) among group II and group I. Prior administration of omega 3 oil (group V, VI and VII) increased the hanging time significantly ($p<0.05$) in 21st and 28th day treatment.

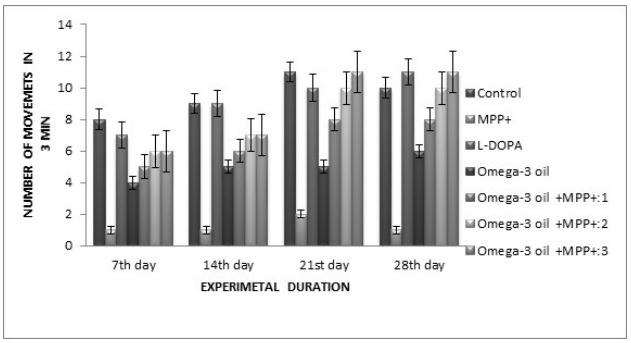

Fig. 4: Shows the rearing movements of drug treated mice

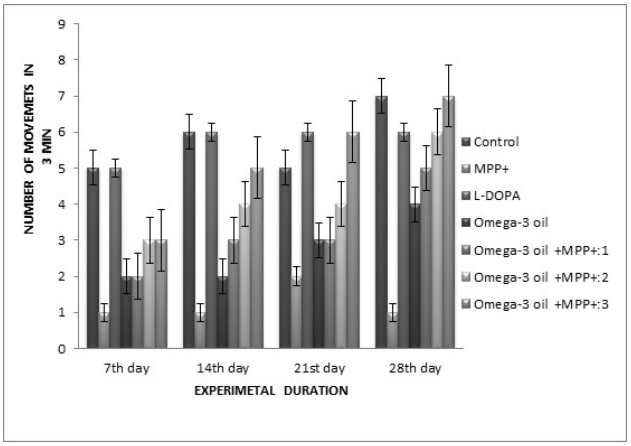

Fig. 5: Shows the grooming movements of drug treated mice

Table 1 and 2 shows the evidence of the biochemical changes of the omega 3 oil administrated mice cortex and midbrains. These tables express the summery of the Lipid peroxidation levels in the midbrain and cortex of control and tested groups. After treatment the lipid peroxidation levels were showed significant increase in group II compared to the control animals (group I) and significant reduction in the omega 3 oil administered animals (group V, VI and VII). In the reduced glutathione assay, the level of decrease was observed in midbrain and cortex region in the MPP^+ alone injected group compared to the control animals (group I) but significant increase was observed in prior treatment of omega 3 oil administered animals

(group V, VI and VII) and group IV didn't show any changes in the TBARS and reduced glutathione levels compared to group I.

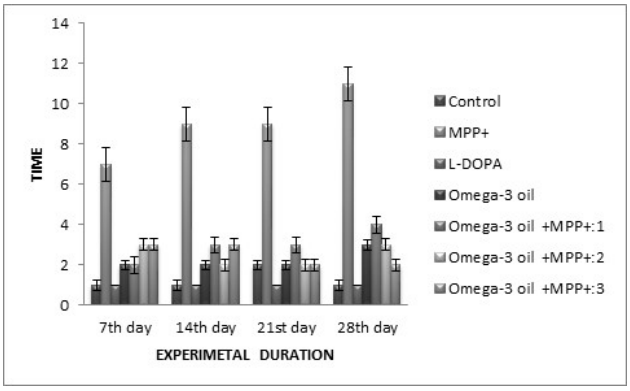

Fig. 6: Effects of Drug Treated mice in Maze Test

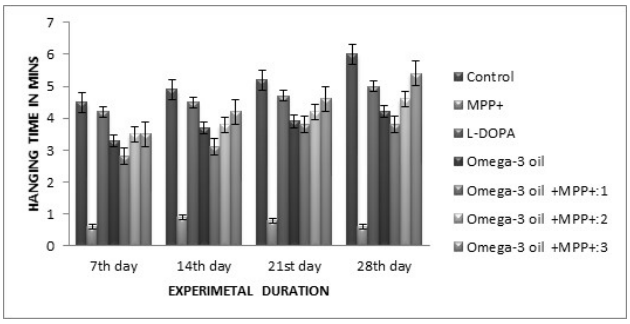

Fig. 7: Effects of drug treated mice in hang test

Control, 2- MPP^+, 3- L-DOPA, 4- Omega-3 oil, 5- Omega3 oil +MPP^+:1, 6- Omega-3 oil +MPP^+:2, 7-Omega-3 oil +MPP^+:3

Fig. 8: SDS-PAGE and Immunoblotting of the drug treated mice

The superoxide dismutase, catalase and glutathione peroxidase assays showed significant decrease in group II (MPP^+ treated animals) compare control group I but omega 3 oil + MPP^+ injected animals (Group V, VI and VII) showed significant increase while compared to Group II. Group IV and Group I didn't showed much change in the SOD, CAT, and GPX levels.

The western blotting carried out to determine the changes of UCH-L1 in PD, we quantified UCH-L1 by Western blotting (fig. 8). Statistical significant difference of UCHL1 expression was found between group II and group I. There was no significant difference in omega 3 oil+ MPP^+ pretreated group (group VII) as compared to control group (group I).

DISCUSSION

This study, clearly indicate the relationship between higher dose level of fatty acid administration and predominance to the PD [16]. In the Rotterdam Study, an approaching population based investigation on 5289 subjects showed that the strength balanced admission of PUFAs reduced the danger of creating PD by 34% (de Lau *et al.,* 2005). Taghizadeh *et al.* (2017) observed reduced in the normal Parkinson's disease rating scale score by supplementation with n-3 polyunsaturated fatty acids and also improved clinical symptoms in PD patients. A case study, notwithstanding, established that dietary admission of n-3 poly unsaturated fatty acids was not prescient for the danger of creating PD.

In the present examination, we evaluated the capability of the OMEGA-3 PUFAs and shield from oxidative weight impelled by MPP^+ on Parkinson's disease in mice. MPP^+ promotes commonly used medicine for induced Parkinson's in animal model. In the investigation the experimental group demonstrated overwhelming outcome as per the portion of the tests. The MPP^+ treated group (Group II) showed low cataleptic score compared to groups V, VI and VII. The highest cataleptic score observed in high dose used test animals (Group VII) in the 28^{th} day compared to other treatment days. These MPP^+-actuated neurotoxicity cause diminished ATP generation, expanded ROS creation and expanded apoptosis of Daergic cells (Blandini and Armentero, 2012). Pretreatment of omega 3 oil on mice (Group V, VI and VII) expressed greater than before peripheral and central activities beside with notable rearing and grooming activities in 14^{th} 21^{nd} and 28^{th} day treatment ($p<0.05$). The behavioral tests such as hang test and open field test normally measured to study the movement on the experimental (Rajasankar *et al.,* 2009).

The open field test, crossing the central squares is a sign of exploratory behavior such as mental stress, adaptation ability and movement activities (Nowakowska *et al.,* 1999). Reduction in the open field spontaneous activity has been reported in MPP^+ treated mice (Group II). Pretreatment of group VIII mice group prevents the MPP^+ toxicity and maintains proper muscular coordination and retains the spontaneous active nature of the mice. In Group III also showed significant reduction in rearing and grooming activities. Besides, the constant administration with omega 3oil in part forestalled the hindrance showed by MPP^+, advancing a significant decrease in rotational behavior and just as expanding the movement activities. This recovery is most likely because of the beneficial impacts of omega-3 poly unsaturated fatty acids weakening neuronal degeneration of dopaminergic cells and diminishing the procedure of modification of dopaminergic receptors (Aguiar *et al.,* 2008). Our results are corroborated with the findings of Binfare *et al*. (2009) who detailed that there is a relative decrease in behavioral movement, raising and all other activities in haloperidol treated animals. The hang test used to determine the strength of neuromuscular (Mohanasundari *et al.,* 2006).

Table 1: The level of TBARS and GSH and activities of SOD, CAT and GPx in midbrain

Group	TBARS *(nmoles/g)*	GSH *(mg/gram tissues)*	SOD *(U^A/mg Protein)*	CAT *(U^B/mg Protein)*	GPx *(U^c/mg Protein)*
Control	0.41±0.6[a]	1.089±0.1[a]	2.34±0.008[c]	1.35±0.005[c]	0.089±0.006[b]
MPP+	0.92±0.2[a]	0.21±0.06[b]	0.556±0.1[a]	0.23±0.17[a]	0.02±0.001[c]
L-DOPA	0.46±0.6[a]	1.12±0.12[b]	2.4±0.19[c]	1.35±0.01[b]	0.081±0.17[a]
Omega-3 oil	0.39±0.12[b]	1.02±0.004[c]	2.22±0.01[b]	1.29±0.33[c]	0.081±0.31[a]
Omega-3 oil +MPP+:1	0.76±0.08[b]	0.66±0.08[b]	0.67±0.3[c]	0.57±0.01[a]	0.042±0.06[c]
Omega-3 oil +MPP+:2	0.69±0.1[a]	0.74±0.15[a]	0.82±0.33[a]	0.89±0.007[c]	0.06±0.09[a]
Omega-3 oil +MPP+:3	0.53±0.01[a]	0.81±0.12[c]	1.2±0.66[b]	1.2±0.11[a]	0.073±0.11[b]

Table 2: The level of TBARS and GSH and activities of SOD, CAT and GPx in cortex

Group	TBARS *(nmoles/g)*	GSH *(mg/gram tissues)*	SOD *(U^A/mg Protein)*	CAT *(U^B/mg Protein)*	GPx *(U^c/mg Protein)*
Control	0.191±0.4[c]	2.68±0.11[c]	3.098±0.01[c]	1.67±0.03[a]	0.097±0.06[c]
MPP+	1.297±0.6[c]	0.26±0.09[c]	0.66±0.11[a]	0.18±0.19[b]	0.02±0.01[a]
L-DOPA	0.21±0.9[a]	2.52±0.19[b]	2.88±0.22[c]	1.45±0.11[c]	0.079±0.19[c]
Omega-3 oil	0.17±0.19[c]	2.53±0.09[a]	3.02±0.11[b]	1.44±0.13[c]	0.081±0.33[c]
Omega-3 oil +MPP+:1	0.56±0.09[c]	1.23±0.03[c]	0.90±0.03[a]	0.77±0.09[b]	0.031±0.16[a]
Omega-3 oil +MPP+:2	0.52±0.11[a]	1.54±0.33[a]	1.82±0.23[b]	0.91±0.01[c]	0.04±0.33[c]
Omega-3 oil +MPP+:3	0.4±0.07[b]	1.81±0.09[a]	1.89±0.36[c]	1.12±0.11[a]	0.051±0.13[c]

A=Amount of enzyme required to inhabit 50%of NBT reduction
B=mmles of H2O2 consumed /min/mg/Protein
C=Amount of Glutathione utilized/minute
Values not sharing a common superscript letter differ significantly at p<0.005 (DMRT).

The significant decrease in droop time was found in L-DOPA injected animals. Pretreatment of omega 3 oil treated animals increased the drooping time significantly. Oxidative pressure and results of lipid per oxidation are ensnared in the pathophysiology of different neuropsychiatric issue. Certain cell reinforcements, for example, melatonin, quercetin, ebselen and Vitamin E have been accounted for to be successful in the treatment of Tardive Dyskinesia (TD), though some ace oxidants, for example, 3-nitropropionic acid and aging spurred TD. These perceptions emphatically bolster the functions of free radicals in TD.

This impact can be connected, at any rate to some degree, to a decrease in explicit endogenous antioxidant mechanisms, for example, a diminishing in reducing glutathione levels and decrease in antioxidant defense enzymes activities like superoxide dismutase and catalase (Elkashef and Wyatt, 1999). In this experiment Table 1 and Table 2 displays the biochemical variation between control and drug treated mice cortex, midbrains. The omega 3 oil administered animals expressed significant reduction in the lipid peroxidation levels. The degrees of reduced glutathione showed low in midbrain and cortex locales in treated groups compared with control group. In TBARS assay, No variations in the level of TBARS and decreased glutathione were observed between group IV and I. Existing proof shows that an unequal creation of free radicals is related through incessant neuroleptic utilize and may add to the beginning of TD and additional development issue, for example, dystonias and Parkinsonism (Cadet, 1986).

Catalase is an antioxidant which assists in neutralizing the dangerous impacts of H_2O_2. H_2O_2 is changed over by the catalase enzyme to from water and non-reactive oxygen species, thus preventing the accumulation of precursor to free radical biosynthesis. Oxidative stress brings about decline in catalase action (Bhangale and Acharya, 2016). MPP^+ injected animals show increase in the oxidative pressure, as indicated by a decrease in the catalase levels. There is no notable changes in the behavior of enzymatic antioxidants were found in omega 3 oil alone compared to control group. There is no much changes found in the omega 3 oil group IV compared to control group. Superoxide dismutase is an enzyme which goes about as an impetus during the time spent dismutation of superoxide into irresponsive oxygen species and H_2O_2. In the experiment the SOD level showed decrease in the brain of MPP^+ administrated mice that implies that oxidative stress is generated. The present outcomes shows that the movements encouraged by omega-3 in the mice model of PD were related with noticeable improvement in the behavioural activities as exhibited from the recovered from Parkinson disorder. This could be credited to the recuperation of DA levels.

CONCLUSION

Discoveries of this investigation exhibit that omega 3 oil exerts neuroprotective impacts in exploratory mice model.

The defensive activities of omega 3 oil exposed in models of MPP^+ neurotoxicity may be credit to its powerful cell reinforcement nature, all things considered; all the properties of this operator might be acting in a purposeful way to deliver neuroprotective reactions.

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