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Monocrotophos treatments induce dopaminergic toxicity in mouse striatum: Evidences from unreported low doses of MPTP

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Abstract
Background: Toxic effect arising from a mixture of chemicals can affect different brain areas thus weakening the overall defense mechanisms and leading to cumulative damage and neuronal death. Using MPTP (as a positive control) at unreported low dose (7 mg/kg b.w), we studied its interactive effect with Monocrotophos (MCP) in different treatment patterns (co-treatment and post-treatment) to ask if a compromised striatum is susceptible to environmental stressors (appearing in low or unreported doses). Evaluations were drawn in terms of neurobehavioral deficits, levels of DA, AChE, markers of oxidative stress and markers of mitochondrial and nitrate stress in the mice striatum.
Methodology: Two treatment patterns of MCP with MPTP were chosen. In the first, MCP was dosed at 0.3 mg / kg b.w on the day 1 along with MPTP (co-treatment) at 7 mg / kg b.w distributed as 4 i.p injections every 2h for 8h on the same day, then followed by MCP oral dosing for 7 days. In the second treatment pattern, MCP was dosed at 0.3 mg / kg b.w for 7 days followed by administration of MPTP (post-treatment) at the above mentioned dose on day 8th.
Results: Post-treatment schedule showed a significant reduction in levels of dopamine, acetylcholine esterase, increased in ROS & LPO, decrease in striatal mitochondrial ATP and complex 1 with no apparent effect on the nitric oxide content.
Conclusion: Our data shows that a compromised striatum is susceptible to environmental stressors that usually appear in low or unreported doses.

Keywords: Dopamine, mice, monocrotophos, parkinson's disease, striatum and behavior

Introduction
Background
Parkinson's disease (PD) is a neurodegenerative disorder that affects primarily the motor skills and cognitive processes (Darweesh *et al*., 2016) [14]. With the advent of the 21st century, its etiology has undergone major restructuring (Darweesh *et al*., 2016; Dorsey *et al*., 2024; Ou *et al*., 2021; van der Gaag *et al*., 2023) [14, 17, 36, 44]. It no longer remains a genetic, age related neurological disorder and many scientists refute genetics as a causative agent as merely 2% cases contribute for such incidences (Duvoisin, 1984; Van Dongen *et al*., 2012) [19, 45]. Furthermore early onset occurrence of the disease gave way to the theory that PD was age related (Kolicheski *et al*., 2022) [33]. Today the gene-environmental interaction theory implicates environmental stressors such as pesticides (Arsuffi-Marcon *et al*., 2024; Kanthasamy *et al*., 2005; Perrin *et al*., 2021) [4, 29], toxic chemical (Dorsey *et al*., 2023; Wan *et al*., 2022) [18, 46] or air pollutants (Calderón-Garcidueñas *et al*., 2021; Cleland *et al*., 2022; Jo *et al*., 2021; Lee *et al*., 2016) [11, 13, 25, 34] as possible contributors to the disease. Over the years, there has been much emphasis on comprehending the adverse effects and the cellular mechanisms that occur during exposure of two or more environmental toxicants in PD pathogenesis (Bogers *et al*., 2023) [7]. Environmental stressors such as pesticides have a global application and demonstrate alterations in a variety of physiological functions known to increases the risk of PD (Dorsey *et al*., 2024) [17].
Low and chronic environmental exposures to a variety of modifiers, mixtures of chemicals and pesticides have been implicated in the alterations of the AChE-DAergic axis in the striatum (Ali, 2020) [1]. Our earlier work provides evidence on the potential of Monocrotophos (MCP) to act as a dopaminergic (DAergic) neutoxicants contributing to the development of PD (Ali & Rajini, 2016) [2]. Besides many organophosphorus insecticides (OPIs) such as Chlorpyrifos (CPF) (Arsuffi-Marcon *et al*., 2024) [4], Diazinon (DI) (Slotkin

& Seidler, 2008) [41] and Dichlorvos (DDVP) (BK *et al*., 2010) [5] have also been implicated in PD like symptoms in rodent models. Low-level prenatal exposure of CPF also leads to reduced IQ scores and deficits in working memory in children (23, 24).s (Kobayashi *et al*., 2017) [32], reported that individuals exposed to chemicals/ neurotoxicants could in the long-run be susceptible to effects such as organophosphate-induced delayed polyneuropathy (OPIDP) or neuropsychiatric disorder (OPIND) (Jokanović & Kosanović, 2010) [26]. The multi-hit hypothesis of neurodegeneration and PD argue about the brains capacity to withstand effects of individual chemical that target the DAergic system. However chronic exposures with a variety of chemical mixtures can target different brain areas weakening the overall defense mechanisms thus leading to cumulative damage and neuronal death (Patrick *et al*., 2019) [37]. Therefore the new approach to study environmental neurotoxicity as possible accelerators of PD should be screening them in relevant combinations as against individual entities. A more holistic understanding of the specific environmental agents that pose greater threat to human health and contribute maximally to the PD pathogenesis is vital for establishing improved regulations and exposure risks.

In a mice models of PD, MPTP is considered to be a powerful drug to induce nigral degeneration and PD-like symptoms at a minimum dose of (14 mg/kg bw) (Bové *et al*., 2005) [8]. We wanted to mimic the interactive effect of low dose MCP with low dose environmental toxicant and so, using MPTP (as a positive control) at unreported low dose (7 mg/kg bwt) in different treatment (co-treatment and post-treatment) scenarios, studied if a compromised striatum is susceptible to environmental stressors (that usually appear in low or unreported doses). We hypothesized that the toxic effects resulting from the mixture of toxicants (pesticide with environmental contaminant) vary in terms of their potency, time of onset and cumulative effects as compared to a single chemical exposure and aggravate the striatal injury which warrants screening them in variable capacities.

Methods

Chemicals

Monocrotophos (Technical grade, 95% pure) was gifted by Hyderabad Chemicals (Hyderabad, India).

Pesticide solution

Monocrotophos (MCP) was dissolved in distilled water and administered orally to mice with a maximum volume of 1.0 ml/kg b.w. for achieving the desired dosage. 1 -methyl- 4-phenyl- 1,2,3,6 -tetrahydropyridine (MPTP) was dissolved in normal saline and administered at a dosage of 7 mg/ kg b.w to mice.

Animals and care

Male Swiss albino mice (25-30 g, 8 weeks old) were used for experimentation. They were housed as three mice per polypropylene cage in a controlled atmosphere with 12:12-hour light/dark cycles, 50% ± 5% humidity and temperature of 25± 2 º C and maintained on a commercial pellet diet (Saidurga Feeds and Foods Pvt. Ltd., Bangalore, India) *ad libitum* with free access to water. All animal procedures were done with approved guidelines from the Institute Animal Ethical Committee. This is regulated by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Social Justice and Empowerment, Government of India.

Preparation of mice striatal homogenate

Mice were euthanized under mild anesthesia and brains were excised and rinsed in cold saline. Isolated striatum were homogenized in either (10%) phosphate buffer saline (100 mM, pH 8.0) or 0.1 M perchloric acid.

Experimental Design

Experiments were conducted on 30 male Swiss albino mice, aged 8 weeks and weighing 25-30g. They were distributed into 5 groups labeled A, B, C, D and E (n=6). The selection of doses of MCP were based on previous studies (Ali & Rajini, 2016) [2]. The total duration for the experiment was 8d with the following experimental design.

Treatments

Group (A) served as the control and received oral gavaging of normal saline.

Group (B) was administered unreported nontoxic dose of MPTP at 7 mg / kg b.w in the form of 4 intraperitoneal (i.p) injections every 2h for over 8h on day 1 (1.75 mg per injection) (Dauer & Przedborski, 2003; Jackson-Lewis & Przedborski, 2007) [15, 23]

Group (C) received MCP orally at a dose of 0.3 mg/kg b.w/d for 7 d - 1/40th dose of the LD_{50} - 12 mg/kg b.w (Chopde *et al*., 1995) [12].

Group (D)-*Co-treatment group*- received nontoxic dose of MPTP at 7 mg / kg b.w in the form of 4 intraperitoneal (i.p) injections every 2h for over 8h on day 1 (1.75 mg per injection) followed by oral gavaging of MCP at 0.3 mg / kg b.w viz., (1/40 of the LD_{50} viz, 12 mg/kg b.w respectively) on the same day itself, followed by MCP dosing till day 8.

Group (E)- *Post treatment group*- 6 mice were administered MCP orally at 0.3 mg / kg b.w - (1/40 of the LD_{50} respectively) for 7 days followed by administration of MPTP (mptp 2) at a dose of 7 mg / kg b.w over 4 i.p injections, every 2h over 8h on the 8th day of the study.

Mice were monitored for neurobehavioral deficits, motor coordination and learning ability for 8 days of experimentation, followed by euthasization on the 9th day of the study.

Analysis of motor coordination and learning - Rotarod test

Motor coordination and learning induced by the treatment was done according to the method of (Deacon, 2013) [16]. Rotarod was used to assess MCP induced neurobehavioral deficits in mice. This was done by measuring the time taken by the animal to remain balanced on the rotating drums. After acclimatize to the experimental conditions, mice were trained on the instrument (Orchid Scientifics, Nasik, India) at an initial speed of 4 rpm, with a constant acceleration of 20 rpm/min. The speed at which the mouse fell off the rod was noted. If a mouse fell off before 10 sec, retrials were given. However if it failed to grip the rod in 10 sec within three consecutive trials, it was assigned a baseline score of 4 rpm. Each animal was subjected to ten trials per day to assess their motor coordination and learning skills.

Estimation of dopamine

The levels of dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were measured by

high performance liquid chromatography (HPLC) with electrochemical detector (ECD) through method of (Gayle *et al*., 2002) [21]. Shimadzu standard system consisted of a high pressure isocratic pump, a 20µl sample injector valve, C18 reverse phase column and electrochemical detector. Data was recorded and analyzed with the help of Shimadzu Class VP version software. Mobile phase consisting of 0.15 M NaH_2PO4, 0.25 mM EDTA, 1.75 mM 1-octane sulfonic acid, and 10% methanol (pH 3.4). Electrochemical conditions for the experiment were +0.600 V, sensitivity ranges from 1 to 100nA. Separation was carried out at a flow rate of 0.5 ml/min. Sample (20 µl) was injected manually. Striatal tissues were homogenized in (10% w/v) 0.1 M perchloric acid, centrifuged at 13,000 × g for 60 min and supernatant filtered through 0.25 µm nylon filters before being injecting into the HPLC system. Data was recorded and analyzed with the help of Class VP version software by Shimadzu. Concentration of DA and DOPAC were expressed as ng/mg protein.

Acetylcholinesterase (AChE) activity

Mice striatum was homogenized (10% w/v) in phosphate buffer saline (PBS, pH 7.8) followed by centrifugation at 10,000 rpm for 10 min at 4 °C. The supernatant was used for assaying acetylcholinesterase activity by method of (Gilani *et al*., 2004) [22]. This is based on the method of (Ellman *et al*., 1961) [20] with slight modifications. Briefly, certain amount of phosphate buffer (100 mM, pH 8) was added with acetylthiocholine iodide (ACTI, 0.01ml of 0.1M) to a mixture containing a suitable amount of striatal homogenate (as a source of enzyme) and DTNB in phosphate buffer (100mM, pH 8). The contents were rapidly mixed and the rate of change in absorbance was monitored over 2 min in a microplate reader at 406 nm. The amount of the enzyme causing a change of 0.001 units of absorbance per minute was considered as one unit of enzyme and the results were expressed as units/mg protein.

Effect on Markers of Oxidative Stress

Reactive Oxygen Species (ROS)

The level of reactive oxygen species (ROS) was quantified using (Keston & Brandt, 1965) [31]. Briefly, tissue homogenate (5%) was incubated with 5µM 2, 7-dichlorofluorescin diacetate (DCFH-DA) in a final volume of 2 ml for 45 min at room temperature. Following the incubation period, fluorescence was recorded at 530nm using the excitation wavelength of 485 nm. The intensity of fluorescence is directly proportional to DCF (resulting from the ROS mediated oxidation of DCFH, which is produced by hydrolytic cleavage of DCFH-DA by cellular esterases). The fluorescence was normalized to protein content and results were expressed as arbitrary fluorescence units/mg protein

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Lipid Peroxidation (LPO)

Lipid peroxidation (LPO) was determined using protocol from (Buege & Aust, 1978) [10]. Briefly 250 µl of the supernatant was added to 2ml of TBA-TCA-HCl solution (thiobarbituric- trichloroacetic acid-hydrochloric acid) (0.374%-15%-0. 25N) and the tubes were placed in boiling water bath for 15 min. After cooling and centrifugation, color of the supernatant was read at 535nm. The amount of thiobarbituric acid reactive substances (TBARS) in the supernatant was calculated using the molar extinction coefficient of 1.56X 10^5 M-1cm-1 and the results were expressed as nmol MDA/mg tissue.

Effect on mitochondrial functions

ATP Content

ATP was determined luminometrically using ATP Determination kit (Molecular Probes, MP 22066 In Vitrogen detection technologies) by method of (Ali & Rajini, 2016) [2]. Striatum was homogenizded (10%) in 0.1 M Tris-acetate-EDTA buffer containing 200mM sodium orthovanadate (ATPase inhibitor) and assayed for ATP content using ATP dependence of the light emitting luciferase-catalyzed oxidation of luciferin. Briefly 20 µl of (10%) striatal homogenate was mixed with 180 µl of "standard reaction mixture" according to the manufacturer's protocol for a total of 200 µl reaction volume then shaken gently and then read in a luminometer to acquire the relative light unit. ATP contents were reported as relative light units / mg protein.

Mitochondrial Complex 1 inhibition

The mitochondrial complex 1 inhibition was quantified by measuring the NADH-cytochrome c reductase activity by method of (Joshi *et al*., 2011) [27]. Briefly, striatal homogenates were homogenized in "Homogenization buffer- (210 mM Mannitol + 70 mM Sucrose+ 5 mM HEPES+ 2 mM EDTA+ 1%BSA)" and spun at 4000-4500 rpm for 15'. The recovered supernatant was centrifuged at 11,000 rpm for 15'. The recovered pellet was dissolved in "Extraction buffer-(210 mM Mannitol+ 70 mM Sucrose+ 5mM HEPES)" and spun at 13,800 rpm for 15' for three cycles. The final pellet was subjected to three freeze thaw cycles of (-22 / 22 °C) and then added with "Hypotonic solution- (5mM HEPES pH 7.4)". At the time of running the assay the final pellet was suspended in 1:1 ratio of Hypotonic and Extraction buffers.

Effect on neuroinflammation

Nitric oxide

The nitric oxide assay was quantified by measuring the amount of nitrite in the striatal homogenates by method of (Schmidt *et al*., 2003) [40]. Briefly, striatal homogenates were homogenized in PBS (pH 7.4) and centrifuged at 10,000 g for 20 minutes. To the supernatant was added *nitrate reductase* at a concentration of (0.8 mg / ml), NADPH (100µM in reaction mixture) and phosphate buffer 7.4 (pH 7.4). This was incubated at room temperature for 2h and then incubated with 100 µl of Griess reagent (50 µl 1% sulfanilamide + 50 µl 0.1% naphthylethylene diamine. The pinkish end point was read on ELIZA reader at optical density (OD) of 540 nm.

Protein estimation

Protein content in brain homogenate was estimated by the method of (Lowry *et al*., 1951) [35] using BSA as standard.

Statistical analysis

Mean and Standard Error values were determined for all the parameters and the results were expressed as Mean ± SE. Data was analyzed employing analysis of variance (ANOVA) followed by post-hoc analysis (Tukey's test) for comparison of means to determine the significance of difference among the groups. "p" values below 0.05 were considered as statistically different.

Results

Effect on motor coordination and learning -Rotarod test

MPTP group (column 2) exposure *per se* did not elicit any significant effect on motor coordination behavior as evident in terms of rounds taken per min and the time spent on the rotarod apparatus (Fig. 1A & B). Likewise mice exposed to MCP group (column 3) also showed no significant alteration on rotarod performance. Further a similar trend in the behavior was evident among mice in the CO group (column 4) when both the toxicants were administered at the same time. Interestingly in the POST group (column 5) mice administered MCP for 7d followed by an acute MPTP dose showed significant change in behavioral test. Column 5 i.e. the POST treatment mice showed a significant decrease in analysis of both the measurements of rounds taken-rpm (38%) and time spent (46%) on the Rotarod performance test.

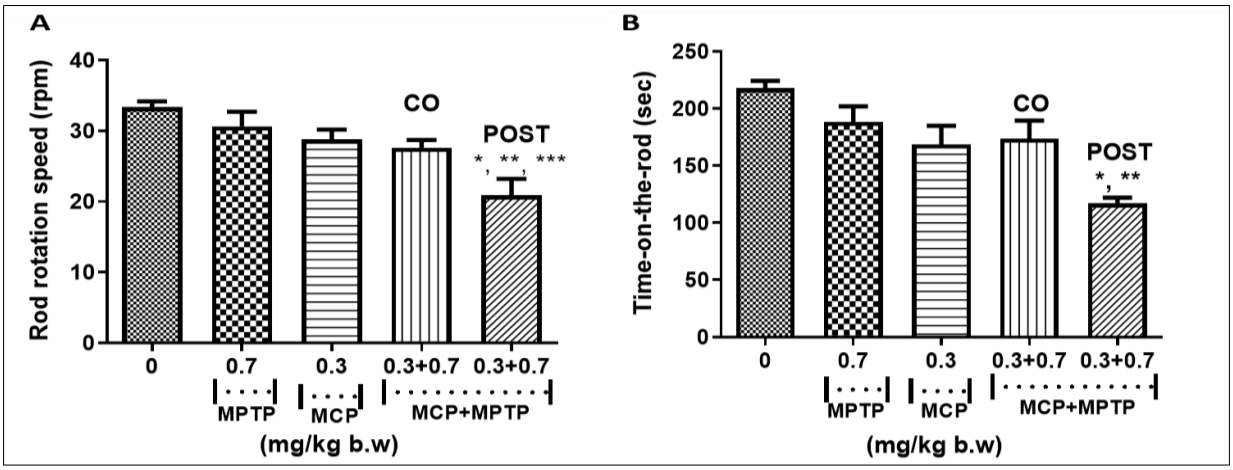

Fig 1: Effect of MCP treatment patterns on the motor coordination and learning using Rotarod test

Values represented are Mean ± S.E (n=6). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test).

(A) Rounds taken per minute on the rod (rpm) * significantly different from MCP ** significantly different from MPTP *** significantly different from Control (p<0.0001).

(B) Time spent on the rod (sec) ** significantly different from MPTP *** significantly different from Control (p<0.0001).

Effect on dopamine, DOPAC levels and AChE activity in striatum

Analysis of dopamine and its metabolite in the striatal homogenates of mice treated with MPTP group (column 2) showed only a marginal decrease in the DA content, while MCP treated group (column 3) showed a higher degree of decrease. These were statistically insignificant however. In the CO treated group (column 4) when mice were administered MCP for 7d along with an acute MPTP dose, there was no significant effect on the decrease in the DA levels again. In contrast however in the POST treated group (column 5) when mice were administered MCP for 7d and then given an acute MPTP dose on the 8th day, a robust decrease (62%) in the DA content was evident (Fig. 2A). A similar trend of results was also observed in the CO group of mice in the DOPAC levels which showed a significantly decrease (78.5%) as compared to MPTP group (column 1) (Fig. 2B). Analysis of AChE levels showed that MPTP group (column 2) alone showed a marginal decrease in the AChE activity. However MCP group (column 3) caused a significant reduction (40%) in the enzyme activity. However mice in the CO treated group (column 4) exhibited a marginal decrease (17%) in the enzyme activity. Interestingly in the POST treated group (column 5) there was a significant decrease (46%) in the AChE activity (Fig. 2C).

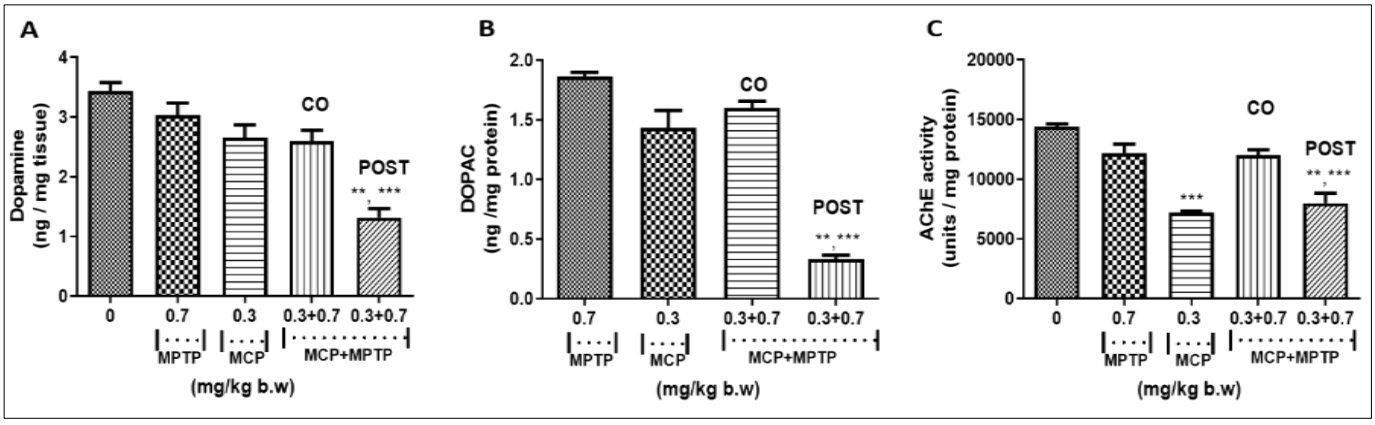

Fig. 2: Effect of MCP treatment patterns on the DA content and AChE activity in mice striatum.

Values represented are mean ± S.E (n=6). Data analysed by ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test).

(A) DA content *** significantly differently from Control and MPTP ** significantly differently from CO ($p<0.0001$)

(B) DOPAC content *** significantly differently from MPTP ** significantly differently from CO ($p<0.0001$) (C) AChE levels * significantly differently from Control ** significantly differently from CO ($p<0.0001$).

Effect on markers of oxidative stress in the mice striatum

ROS & LPO

MPTP group (column 2) exposure *per se* did not elicit any significant effect on the levels of ROS as evident in terms of the intensity of fluorescence is directly proportional to DCF (resulting from the ROS mediated oxidation of 2, 7-dichlorofluorescin diacetate, which is produced by hydrolytic cleavage of DCFH-DA by cellular esterases) which was merely (12% increase as compared to control). Likewise mice exposed to MCP group (column 3) also showed no significant alteration as compared to control group (28% increase). Similarly the CO group (column 4) was also insignificant in the generation of ROS levels with merely (15% increase as compared to control). The POST group (column 5) mice however showed significant change in the levels of ROS generated. POST group showed * significance as compared to the MPTP group and the CO treatment group separately. Furthermore it showed ** significance as compared to the control group with (69% increase of ROS levels) (Fig. 3A)

MCP group (column 3) was significant alteration as compared to control group in terms of the levels of LPO as evident in terms of the amount of thiobarbituric acid reactive substances (TBARS) generated in the supernatant (40% increase as compared to control). The CO group (column 4) was insignificant as compared to control. The POST group (column 5) mice however showed significant change in the levels of LPO generated. POST group showed * significance as compared to the CO treatment group, ** significance as compared to MPTP group and *** significance as compared to the control groups separately (45% increase as compared to control) ($p<0.0001$) (Fig. 3B).

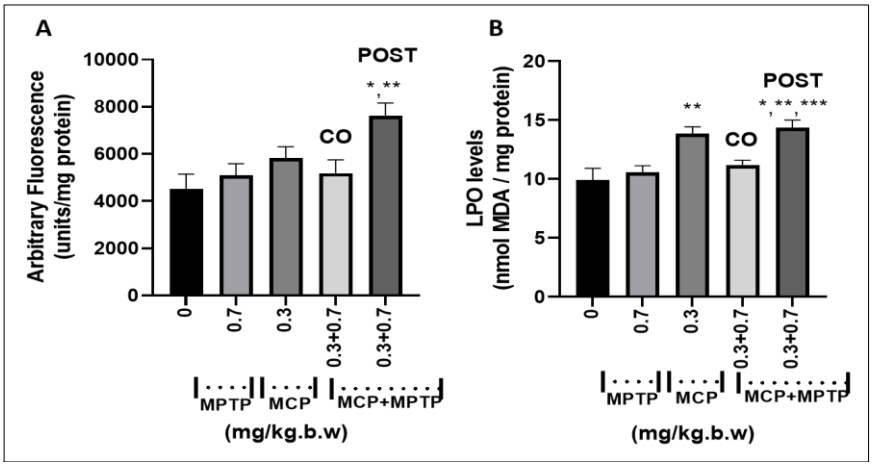

Fig 3: Effect of MCP treatment patterns on the markers of oxidative stress in mice striatum.

Values represented are Mean ± S.E (n=6). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test).

(A) ROS levels * significantly different from MPTP and CO ** significantly different from Control ($p<0.0001$).

(B) LPO levels * significantly different from CO ** POST significantly different from MPTP ** MCP significantly different from Control *** significantly different from Control ($p<0.0001$).

Effect on markers of mitochondrial function in the mice striatum

ATP Content & Mitochondrial Complex 1 inhibition

MPTP, MCP, CO and POST treatment groups all show *** significance in decrease in the ATP levels as compared to control group. Interestingly the MPTP arm (column 2) showed a difference of *** significance as compared to the POST treatment arm (column 5) (MPTP showed 25% decrease whereas POST showed 60% decrease in the ATP levels as compared to the control arm). Between MCP (column 3) and the POST group (column 5), MCP showed around (57% decrease) and POST arm (60% decrease) in the the ATP levels. Similarly the CO group (column 4) was insignificant with merely (33% decrease as compared to MCP). Furthermore the CO vs POST groups showed *** significance ($p<0.0001$) (Fig. 4A).

MCP (column 3) and POST treatment (column 5) groups show ** & *** significance in decrease in the mitochondrial complex 1 inhibition as compared to control group. Interestingly the MPTP (column 2) and POST (column 5) arms showed a difference of * significance in the decrease of complex 1 activity (MPTP showed 13% decrease whereas POST showed 36% decrease as compared to the control arm). Between MCP (column 3) and the POST group (column 5), MCP showed an insignificant decrease around (25% decrease) and POST arm (35% decrease) between them as shown in (Fig. 4B) ($p<0.0001$).

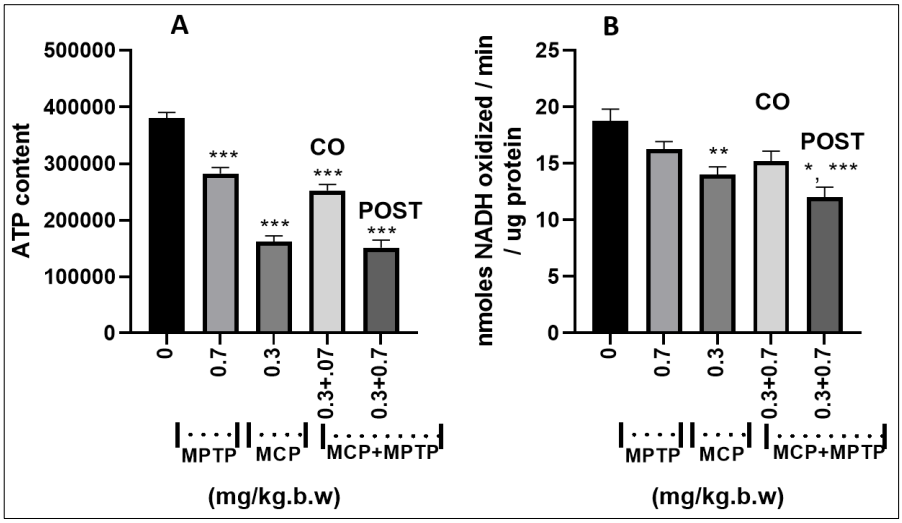

Fig 4: Effect of MCP treatment patterns on the markers of mitochondrial functions in mice striatum.

Values represented are Mean ± S.E (n=6). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test).

(A) ATP content *** MPTP, MCP, CO, POST significantly different from Control *** MPTP significantly different from POST ***CO significantly different from POST (p<0.0001).

(B) Mitochondrial Complex 1 inhibition * significantly different from MPTP ** significantly different from Control *** significantly different from Control (p<0.0001).

Effect on neuroinflammatory marker in striatum

Nitrite levels: Neither MCP nor MPTP treatment patterns induced any change in the nitrite levels in the striatum as evident from (Fig. 5).

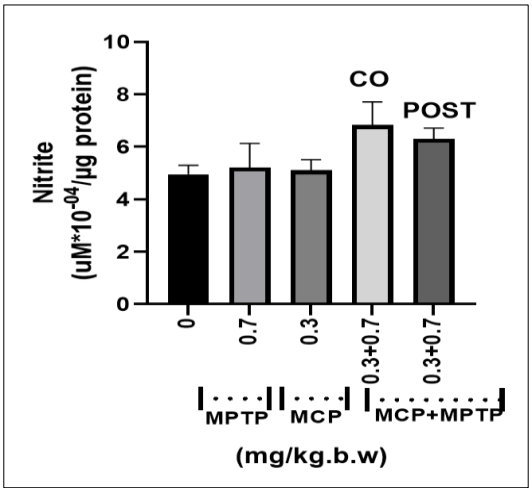

Fig 5: Effect of MCP treatment patterns on marker of neuroinflammation in mice striatum.

Values represented are Mean ± S.E (n=6). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test).

Nitrite levels showed no significant difference in treatment arms (p<0.0001).

Discussion

We wanted to mimic the interactive effect of low dose MCP with low dose environmental toxicant and so, using MPTP (as a positive control) at unreported low dose (7 mg/kg bwt) in different treatment (co-treatment and post-treatment) scenarios, studied if a compromised striatum is susceptible to environmental stressors (that usually appear in low or unreported doses). Evaluations were drawn in terms of neurobehavioral deficits, levels of DA, AChE, markers of oxidative stress and markers of mitochondrial and nitrate stress in the mice striatum.

In the neurobehavioral studies, we evidenced the clinical feature of muscle rigidity, tremors and bradykinesia consistent with previous reports of neurobehavioral deficits in mouse model (Trancikova *et al*., 2011) [43]. Inferences in our study were drawn by comparing the results of the rotarod scores of the MCP treatment to induction of waxy rigidity and deficits of motor coordination in mouse which could be because of brain damage involving parts of the basal ganglia. In our study, mice in the MCP, MPTP and CO treatment groups did not show any difference to control or within the groups. Interestingly in the POST treatment group mice administered MCP for 7d followed by an acute MPTP dose showed significant decrease in analysis of both the measurements of rounds taken-rpm (38%) and time spent (46%) on the rotarod performance test, which clearly suggested functional deficit in motor coordination from loss of muscle tone from neuromuscular blockade and hypokinesia that were produced after MCP exposures in the second treatment group. (Fig. 1&2).

Previously we have reported that mice treated with low toxic doses of MPTP (14 mg/kg b.w) showed significant catalepsy deficits (51%), that were comparable to low dose of MCP exposures (0.6 mg/kg b.w) and argued that the decreased DA levels in the striatum caused these symptoms to appear (Ali & Rajini, 2016) [2]. A similar comparable trend was also seen in our work with *C. elegans* (Jafri Ali & Sharda Rajini, 2013) [24] where we observed a significant decrease in locomotion (in terms of body bends) at much lower concentrations of MPTP than was previous reported (Braungart *et al*., 2004) [9]. Here in this study as compared to CO treated group the POST treated group showed a robust decrease (62%) in the DA content as evident (Fig. 2A). A similar trend of results was also observed in the CO group of mice in the DOPAC levels which showed a significantly decrease (78.5%) as compared to MPTP group (Fig. 2B). Analysis of AChE levels showed that MPTP group alone showed a marginal decrease in the AChE activity. MCP

group caused a significant reduction (40%) in the enzyme activity, as it is an AChE inhibitor. However mice in the CO treated group exhibited a marginal decrease (17%) in the enzyme activity. Interestingly the POST treated group showed a significant decrease (46%) in the AChE activity (Fig. 2C). Similar trends have been studied using pesticides like DDVP and MCP that display competitive nature in inhibiting the AChE activity when given individually. However no synergism happened when the two were given together. Likewise it could be hypothesized that in our study, the chemical interaction in the CO treatment schedule was either antagonistic or low synerginism due to competitive inhibition. It could be that MCP and MPTP competed with each other for the same active binding site, which resulted in a higher affinity of the substrates for the same site.

Striatum has a dense DAergic innervation in the mammalian brain with substantia nigra (SN) and the ventral tegmental area (VTA) being the main contributors. The striatum is also complimented with dense innervations of local, tonically active cholinergic interneurons that release acetylcholine (Threlfell *et al*., 2012) [42]. There was also a dense expression of tyrosine hydroxylase (TH), choline acetyl transferase (ChAT) and acetylcholineesterase (AChE) activity. OPI exert their toxicity primarily by inhibiting the activity of AChE in the brain (Kazi & Oommen, 2012). On reaching the nerve synapses, they block the hydrolysis of the bound acetylcholine (ACh). This causes the post-synaptic membrane to be over stimulated which in turn induces excitability, tremors, paralysis and finally death. In the present study we found that MCP showed a significant reduction in AChE activity (40%). On comparing the first and the second treatment schedule of MCP with MPTP, we found a significant decrease of (46%) in AChE activity in the second treatment schedule. The similarity in decrease in both the DA and the AChE content in POST treatment group could be due to the synchronized activity in the cholinergic inter neurons directly generating striatal DA signals, that is, synchronized activity in the striatal cholinergic interneurons also trigger DA signals (Threlfell *et al*., 2012) [42]. These findings corroborate with previous reports (Zhou *et al*., 2001) [47] which have shown that the endogenous nicotinic cholinergic activity regulates DA release in the striatum. Previous reports have also demonstrated that the DA blockade by neuroleptics leads to increased release of ACh in the striatum (Chopde *et al*., 1995) [12].

Oxidative stress is essential to understanding the mechanism of tissue damage (Kannan & Jain, 2000) [28] which could lead to the generation of ROS and LPO. Similarly the mitochondria have an important role in the pathogenesis of PD as is evident from the observation that MPP+, which is the active metabolite of MPTP, causes an inhibition of complex 1 of the mitochondrial electron transport chain (ETC). These changes could be speculated as the molecular mechanisms involved in OPI-induced toxicity (Altuntas *et al*., 2002) [3]. (Binukumar *et al*., 2010) [5] reported that DDVP showed a positive correlation in terms of generation of ROS, and LPO activity. Similarly in our study, we observed that the POST treatment group further showed significant increase in the levels of ROS, LPO and concomitant decrease in terms of the total ATP and Complex 1(NADH-dehydrogenase) activity levels. Dichlorvos has also been reported to potentiate ATP degeneration and complex I inhibition in the mitochondria in rats and concomitantly reduce dopamine levels in the SN and CS (Binukumar *et al*., 2010) [5]. The striking insignificance between the CO and the POST treatment groups could be attributed to hormesis like phenomenon. In the CO treatment group, was a modest increases in ROS levels activating a positive cellular response? Furthermore our study showed no change in the nitrite levels (a marker of neuroinflammation), similar to studies carried out by other researchers (Binukumar *et al*., 2010) [5].

Conclusion

This is a preliminary finding and further studies at molecular and docking levels might be able to further substantiate the hypothesis that a compromised striatum is susceptible to environmental stressors that usually appear in low or unreported doses.

List of Abbreviations

Monocrotophos (MCP), dopaminergic (DAergic) Parkinson's disease (PD), organophosphorus insecticides (OPIs), Chlorpyrifos (CPF), Diazinon (DI), Dichlorvos (DDVP), organophosphate-induced delayed polyneuropathy (OPIDP) neuropsychiatric disorder (OPIND) dopamine (DA), 3,4- dihydroxyphenylacetic acid (DOPAC), high performance liquid chromatography (HPLC), electrochemical detector (ECD), reactive oxygen species (ROS), lipid peroxidation (LPO)

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Author's contribution

Shaheen Jafri Ali and Kisan B Jadhav contributed to the study's conception, design, and drafting of the manuscript. Md. Touseef Khan was responsible for developing the study methodology, material preparation and data collection. All authors commented on previous versions of the manuscript during the review and editing stages. All authors read and approved the final manuscript. Shaheen Jafri Ali acquired funding for the study.

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Availability of data and material

On request.

Declarations

Conflict of interest

The authors of the article state that they have no conflict of interest.

Ethics approval

All animal procedures were done with approved guidelines from the institute animal ethical committee. This is regulated by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), under the ministry of social justice and empowerment, government of India.

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