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Research Article

Zinc and Linoleic Acid Protect Against Behavioural Deficits In Rat Model Of Parkinsonism Induced With Rotenone

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ABSTRACT

Little is known about the behavioural effect of either Zinc or Linoleic acid or their combination in the delay of onset of Parkinson's disease. This study was designed to investigate the effects of Zinc and Linoleic acid in the protection of behavioural deficits in rotenone-induced Parkinsonism in rats. Thirty six young adult female rats weighing 100-150 grams divided into six groups were used. Rats were induced with Parkinsonism by subcutaneous administration of rotenone (Sigma-Aldrich, St. Louis, SA) (2.5mg/kg) once a day for seven consecutive days. Rats received (Dimethyl sulfoxide) DMSO/Olive oil or rotenone dissolved in DMSO/Olive oil. Groups III and IV received Zinc (30mg/kg) or Linoleic acid (150µl/kg) while group V received a combination of both, two weeks prior to rotenone injection. Groups II and VI served as negative (rotenone group) and positive (Levodopa groups) controls respectively. Measurement and analysis of behavioural function in rats employed a battery of tests including Elevated Plus Maze (EPM), Open Field Test (OFT), Novel Scent and Block Tests. Rats receiving rotenone displayed bradykinesia and motor impairment in the OFT, anxiety, decrease in olfactory acuity and discrimination in EPM, and Novel Scent Test respectively. The significant increase in postural instability, impaired motor activity/coordination, increased anxiety and the decrease in rearing behaviour caused by rotenone induction was attenuated significantly by treatment with Zinc and Linoleic acid, but not their combination. These results suggest that Zinc possess significant behavioural activity while Linoleic acid improves certain aspects of sensory and motor function than their combination.

Keywords: Anxiety, antioxidant, olfactory deficit, sensorimotor assessment, ageing

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INTRODUCTION

Parkinson's Disease, is the second most common neurodegenerative disorder with an incidence of around 14 per 100,000 population worldwide (Blanckenberg *et al.*, 2013) and roughly 50,000 new cases arise every year. Parkinsonism refers to a clinical syndrome characterized by a variable combination of rest tremor, bradykinesia or akinesia, rigidity, and postural instability (Elbaz *et al.*, 2016; Prema *et al.*, 2015). Other non-motor symptoms include anxiety, depression, sleep alteration, gastrointestinal dysfunction and olfactory disturbances which are one of the first non-motor symptoms observed in PD patients (Tillerson *et al.*, 2006).

Current treatment of Parkinson's disease (PD) is based on dopamine replacement therapy, but chronic administration may cause motor fluctuations and dyskinesias, increased free radical formation, and may not address most of the non-motor symptoms (Dotchin, Msuya, & Walker, 2007). Several agents have been investigated for the management of behavioural deficits in Parkinson disease, yet none has been largely successful. Zinc has been used in the management of diarrhoea (Abdullah *et al.*, 2006), acute lower respiratory infections (Sazawal *et al.*, 1998) and male infertility (El-Tawil, 2003). Linoleic acid, a potential nutraceutical has been shown to remediate behavioural deficits resulting from stroke (Iso *et al.*, 2002) and dementia (Sydenham, Dangour Alan, &

Lim, 2012), yet their protective effect against the development of behavioural deficits in PD has not been investigated. The choice of Zinc for its use in PD is motivated by significant low levels of Zinc in the cerebrospinal fluid (CSF) of PD patients in several studies (Frederickson, Koh, & Bush, 2005). This suggests that Zinc deficiency may accompany many cases of PD. Chronic Linoleic acid deficiency significantly decreases storage formation of vesicles that store dopamine (Dyall & Michael-Titus, 2008; Eckert, Lipka, & Muller, 2013).

Evidence suggests that Zinc increases synthesis of metallothionein, an antioxidant which plays an important role in protecting dopaminergic neurons from free radical damage and environmental neurotoxins (Lehto *et al.*, 2013), it also stabilizes the cell membrane. Linoleic acid modulates dopaminergic, serotonergic and cholinergic neurotransmission and the transcription of genes involved in inflammation, oxidative stress and neurotrophic support (Zimmer *et al.*, 2000). Using the hypothesis that micronutrient and trace element deficiency is implicated in mitochondrial dysfunction and oxidative injury underlie neurodegeneration in PD (Eckert *et al.*, 2013; Joshi, Mittal, Shukla, & Srivastav, 2012; Tõugu & Palumaa, 2012); the inclusion of metabolic modifiers may provide an alternative and an early intervention approach. In order to investigate this we used a toxin-induced model of Parkinson's disease to assess the protective effect of Zinc and Linoleic acid against development of behavioural deficits in PD patients. These agents may provide a potential therapy aimed for use as pharmacologic interventions to protect against behavioural deficits in PD.

MATERIALS AND METHODS

Chemicals and drugs: Zinc dust, linoleic acid and rotenone were obtained from Sigma-Aldrich, St Louis, SA. 125 mg of rotenone was dissolved in 1ml of DMSO to prepare a 50X stock solution of rotenone in 100% DMSO. 40µl of the stock solution (125 mgs/ml) was then diluted in 1960 µl of olive oil. The solution was vortexed to create a stable emulsion of the DMSO containing rotenone and Olive oil. The solution was made fresh 2-3 times/week and stored in an amber septa vial protected from light and inverted several times before each injection to eliminate the possibility of settling. The solution was administered at a volume of 1 ml/kg and control animals received the vehicle only (Olive oil/DMSO) (Ojha *et al.*, 2015). All other chemicals and reagents were obtained from the Biochemistry and Physiology laboratories of the University of Yaounde 1 Cameroon and were of analytical grade.

Animals: Female Wistar rats were used in the present study. Their weights ranged between 100 and 150 g. The rats were housed in cages under clean laboratory conditions at room temperature with reversed light and dark cycles. Water and food pellets were given *ad libitum*. All the experimental protocols were approved by the Institutional Animal Care and Ethics Committees of the University of Yaounde 1, Cameroon under the reference number N° 2017/01/699/CE/CNERSH/SP.

Experimental design: Thirty six young adult female rats aged 8-12 weeks and weighing 100-150 gm obtained from the animal house, of the Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Cameroon and acclimated at room temperature of $25 \pm 1^\circ\text{C}$ (Hawkins, 2002) were used (Gupta *et al.*, 1986). Parkinsonism was induced using 2.5 mg/kg dose of rotenone subcutaneously (Ojha, Javed, Azimullah, Khair, & Haque, 2015). Rotenone was administered at a volume of 1 ml/kg body weight.

A group distribution flowchart detailing the different treatments and route of administration used in the study is shown below..

Table 1:
Experimental Design

Group (n=5)	Intervention Received	Dosage	Duration (days)	Route
I	Olive oil/DMSO	1 ml/kg	21	PO
II	Rotenone	2.5mg/kg	7 last days	SC
III	Zinc dust + rotenone	30 mg/Kg + 2.5 mg/kg	21 days + 7 last days for rotenone	PO + SC (rotenone)
IV	Linoleic acid + rotenone	150 ug/kg + 2.5 mg/kg	21 days + 7 last days for rotenone	SC
V	Zinc + linoleic acid + rotenone	30 mg/kg + 150 ug/kg + 2.5 mg/kg	21 days + 7 last days for rotenone	PO + SC
VI	Levodopa/ Carbidopam + rotenone	6mg/Kg + 2.5 mg/kg	Last 7 days	PO + SC

PO= oral; SC = subcutaneous

Behavioural assessment: Twenty four (24) hours after the last injection of rotenone, rats were screened for behavioural deficits. Behavioural function in rats was measured using a series of tests including; the elevated plus maze which assessed anxiety behaviour, open field test assessed exploration and locomotor ability as well as anxiety. The novel scent and block tests assessed olfactory acuity and olfactory discrimination. Data collection instruments included a stop watch timer, video recorder, cages, and vanilla syrup, small blocks, elevated plus maze and wooden open field box. The rats were coded and behavioural assessment done by an independent trained experimenter blinded to groupings and interventions.

Locomotor Assessment: The Open Field Test provides a means for measuring, exploration, locomotion and anxiety in rodents. The dimensions of the open field maze were 72 x 72 cm with 36 cm walls. The large open field was used for measuring exploration as well as locomotion (Walsh & Cummins, 1976). Rats were placed into the centre or at the corners and were allowed to explore the space for minutes. The ambulation and rearing frequencies were used as measures of locomotor activity, but also measures of exploration and anxiety (Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002; Gould, Dao, & Kovacsics, 2009). Grooming and freezing were used as

measures of exploration and anxiety. The horizontal movement (ambulation frequency and freezing) and the vertical movement (rearing frequency) were registered (Stanford, 2007).

Anxiety Assessment: Anxiety assessment was done using the elevated plus maze (Hogg, 1996). The time spent in the open and closed arms are assessed by this test and are used as measures of anxiety. Behaviour in this task reflects a conflict between the rodent's preference for protected areas and their innate motivation to explore novel environments. The elevated plus maze relies upon rat's inclination toward dark, enclosed spaces and a natural fear of heights and open spaces (Carola *et al.*, 2002; Hogg, 1996; Holmes, Parmigiani, Ferrari, Palanza, & Rodgers, 2000). The EPM consisted of two open arms without walls and two arms closed by walls about 30cm high. The arms were 50 cm long and 10 cm wide. Each rat was placed at the junction of the four arms and number of entries and the total duration of entries into each arm were recorded for 5 minutes. The maze was cleaned with 70% alcohol and dried. The behavioural parameters recorded were: numbers of entries into the open and closed arms; time spent in the open and closed arms. Open arm time was used as an index of anxiety-like behaviour, while number of entries in closed arms was considered as a measure of locomotor activity (Pellow, Chopin, File, & Briley, 1985). A rat was considered to have entered an arm when all its four paws were on that arm.

Assessment of olfactory acuity: Disturbances in sense of olfaction are one of the earliest non-motor symptoms observed in PD and patients. These include deficits in odour detection, differentiation, and identification (Tillerson *et al.*, 2006). Olfactory acuity was measured using the novel scent test, which is a simple way of quantifying time spent sniffing a new odour (Doty, 2012; Doty, Shaman, Kimmelman, & Dann, 1984). Each rat was presented small quantities of both vanilla and water simultaneously (Taylor *et al.*, 2009). Time spent sniffing each odour was recorded for a three minute session. Time spent sniffing the vanilla indicates functional olfactory system to detect new odours. The frequency of sniffing indicates exploratory ability to novelty.

Assessment of olfactory discrimination: The block test was used to evaluate the ability of rats to discriminate between social odours, specifically self and non-self (Taylor *et al.*, 2009). Each rat was presented with a block smeared with its own faecal matter and a block smeared with faecal matter of another rat of the same sex. The time spent in contact with each block was recorded for a two minute trial. More time spent sniffing the bedding of another rat indicated social attraction, cohesion and novelty, all of which points to the rat's ability to discriminate between self from non-self as well as social groups. This ability is crucial for feeding, social communication and mating. It is also crucial for the process of learning and memory that come along with these behaviours.

Statistical analysis: Results were collected, tabulated and expressed as mean \pm S.E.M. Measurements were analyzed using one-way analysis of variance, ANOVA, followed by Tukey's multiple comparisons test. All statistical tests were

done employing Graph Pad Prism version 6. Differences were considered significant at $p \leq 0.05$

RESULTS

Administration of rotenone (2.5 mg/kg) subcutaneously for seven days consecutively produced anxiety-like behaviors in the elevated plus maze, bradykinesia and postural instability in the open field test.

Assessment of behavioural function: Treatment with Zinc, Linoleic acid or their combination ameliorated to varying degrees the behavioural deficits due to administration of rotenone.

Locomotor Assessment: There was a significant decrease in the locomotor activity, as expressed by decreased ambulation frequency and rearing, in case of rotenone group when compared with control group ($P < 0.05$)

Latency of movement initiation: Rotenone-treated rats showed increased latency to initiate movement as compared to the control rats ($p < 0.05$). Zinc, Linoleic acid and their combination reduced the latency time as compared to rotenone-treated group ($p < 0.05$). The effect of Linoleic acid was also significantly higher when compared to vehicle group ($p \leq 0.05$, Figure 1).

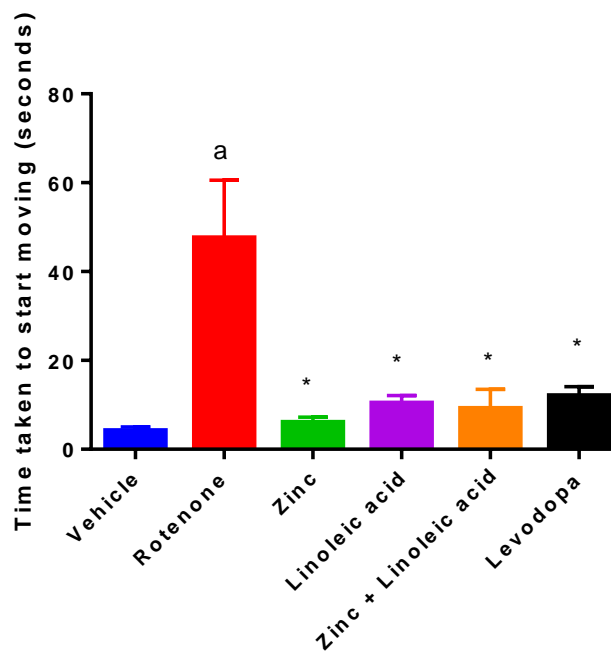


Figure 1: Effect of Zinc, Linoleic acid and their combination on the latency for initiation of movement by rats. a, $p \leq 0.0001$ compared to vehicle group, * $p \leq 0.0001$ compared to rotenone group.

Ambulation frequency

The present results showed a significant reduction in frequency of ambulation in rotenone group as compared to the normal control group (Fig. 2). Zinc, significantly increased the ambulation

frequency as compared to rotenone group ($p < 0.0001$). Neither Linoleic acid nor the combination of Zinc and Linoleic acid significantly increased the ambulation frequency compared to the rotenone group ($p > 0.05$, Figure 2)

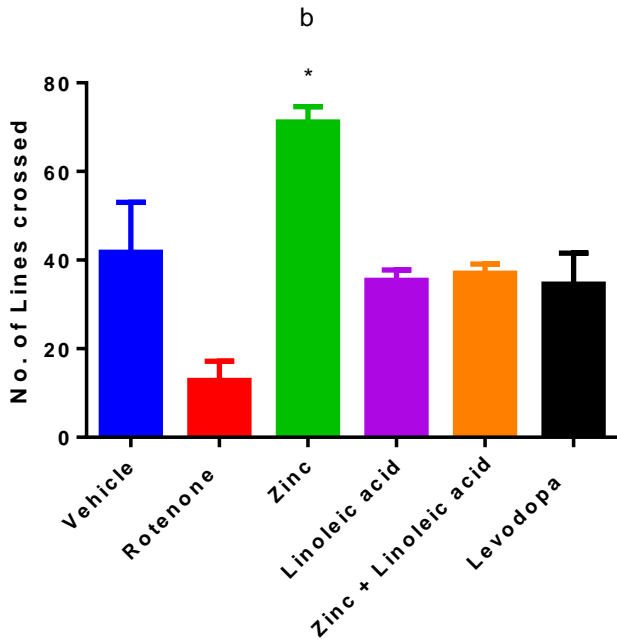


Figure 2: Effect of Zinc, Linoleic acid and their combination on the ambulation frequency of rats. *, $p \leq 0.0001$ compared to vehicle group, b $p \leq 0.05$ compared to Levodopa

Freezing Duration: The duration of inactivity recorded as the freezing was significantly higher in rotenone-treated animals compared to the control group. Treatment with Zinc and Linoleic acid and their combination significantly reduced the freezing duration in comparison to the rotenone group ($p \leq 0.0001$, Table 2 and Figure 3)

Grooming Duration: Rotenone-treated rats showed increased grooming duration as compared to the normal control group. Zinc and their combination but not Linoleic acid alone significantly decreased the grooming duration as compared to rotenone group ($p \leq 0.0001$ and $p < 0.05$ respectively, Table 2 and figure 4).

Rearing frequency: The rotenone group showed significantly lower frequencies in rearing compared to the vehicle group ($p < 0.0001$) (Table 2 and figure 5). In addition, the current results indicated that Zinc and Linoleic alone but not their combination significantly increased the rearing frequency compared to rotenone group ($p \leq 0.0001$ and $p < 0.05$ respectively). The effect of Zinc ($p < 0.0001$) and Linoleic acid ($p < 0.05$) on the rearing behavior was significantly higher when compared to Levodopa.

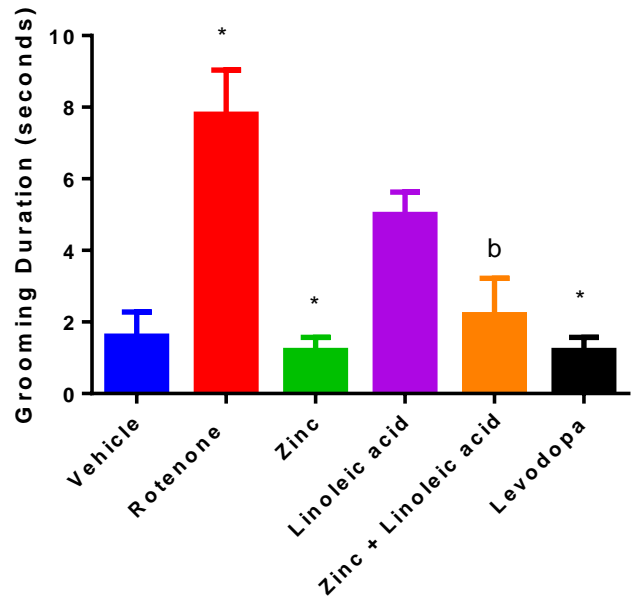


Figure 4: Effect of Zinc, Linoleic acid and their combination on the grooming duration of rats. *, $p \leq 0.0001$ compared to rotenone group; b $p < 0.05$ compared to rotenone group

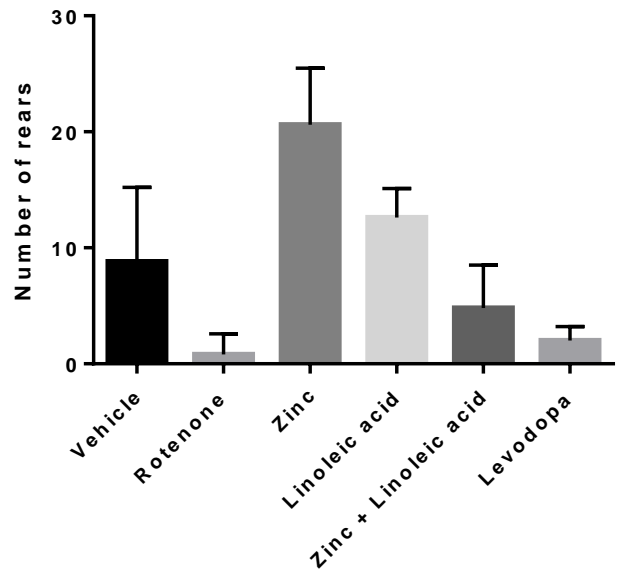


Figure 5: Effect of Zinc, Linoleic acid and their combination on rearing frequency of rats. *, $p \leq 0.0001$ compared to rotenone group; b $p < 0.05$ compared to rotenone group

Anxiety Assessment: In the elevated plus maze, the rotenone-treated group significantly spent less time in the open arm compared to the vehicle-treated group ($p < 0.05$, Table 2). Zinc but not Linoleic acid or their combination significantly increased the number of entries into the open arms of the maze. compared to the rotenone-treated group ($p < 0.0001$). Zinc and its combination with Linoleic acid but not Linoleic acid alone significantly decreased the number of entries into

the close arms of the maze compared to the rotenone-treated group ($p < 0.05$). Zinc and Linoleic acid but not their combination increased the rearing frequency.

Assessment of Olfactory acuity

Rotenone-treated rats showed decreased sniffing time as compared to the vehicle-treated rats. Zinc and Linoleic acid and their combination significantly increased the sniffing duration of vanilla as compared to rotenone group ($p \leq 0.0001$ Table 3). The sniffing of water by the rotenone-treated group was significantly decreased ($p < 0.05$, Table 3) as compared to the vehicle-treated group. The frequency of sniffing vanilla was also significantly decreased in

the Zinc, Linoleic acid and the combination group when compared with the rotenone group.

Assessment of Olfactory Discrimination

Rotenone-treated rats showed significant decreased sniffing time of non-self-bedding as compared to the vehicle-treated rats ($p < 0.0001$). Zinc and Linoleic acid and their combination significantly increased the sniffing duration of non-self bedding as compared to rotenone group ($p \leq 0.0001$ Table 4). In addition, Zinc and Linoleic acid and their combination significantly increased the frequency of sniffing non-self-bedding as compared to rotenone group ($p \leq 0.0001$ Table 4).

Table 2:

Effect of Zinc and Linoleic acid and their combination on parameters of elevated plus maze

Groups	Open Arm Time (seconds)	Close Arm Time (seconds)	Open Arm Entries	Close Arm Entries
Vehicle	52.40 ± 9.626	129.2 ± 27.52	4.000 ± 0.5477	4.800 ± 1.158
Rotenone	15.00 ± 2.775	239.6 ± 21.45 _a	1.800 ± 0.3742 _a	3.200 ± 0.6633
Zinc	180.6 ± 18.50*	42.60 ± 2.502*	7.200 ± 0.3742*	5.600 ± 0.2449
Linoleic Acid	32.00 ± 4.680	186.4 ± 17.69	3.400 ± 0.5099	6.000 ± 1.095
Zinc + Linoleic Acid	56.60 ± 17.44*	117.6 ± 32.11*	3.000 ± 0.7071	3.600 ± 0.6782
Levodopa	50.20 ± 8.558*	57.80 ± 7.566*	4.200 ± 0.3742 _b	5.200 ± 0.7348

Results are expressed as mean ± SEM. *a*, $p \leq 0.05$ compared to vehicle group; *b*, $p \leq 0.05$ compared to rotenone group; * $p \leq 0.0001$ compared to rotenone group

Table 3:

Effect of Zinc and Linoleic acid and their combination on parameters of novel scent test

Groups	Duration of sniffing water (seconds)	Duration of sniffing vanilla (seconds)	Freq. of sniffing water	Freq. of sniffing vanilla
Vehicle	25.00 ± 3.536	93.20 ± 4.576	2.000 ± 0.3162	4.200 ± 0.3742
Rotenone	15.00 ± 2.588 _a	7.600 ± 3.203 _c	3.600 ± 0.4000	0.8000 ± 0.3742 _a
Zinc	18.40 ± 1.288	93.00 ± 5.385*	1.800 ± 0.3742	5.600 ± 0.9274*
Linoleic Acid	7.600 ± 1.536 _b	81.00 ± 5.486*	1.800 ± 0.3742	6.600 ± 0.7483*
Zinc + Linoleic Acid	9.200 ± 2.458	94.00 ± 7.190*	2.600 ± 0.5099	7.000 ± 1.049*
Levodopa	11.00 ± 0.7071	72.00 ± 4.626*	2.200 ± 0.3742	5.800 ± 0.7348

Results are expressed as mean ± SEM. *a*, $p \leq 0.05$ compared to vehicle group, *b*, $p \leq 0.05$ compared to rotenone group; *c* $p \leq 0.0001$ compared to vehicle group * $p \leq 0.0001$ compared to rotenone group

Table 4:

Effect of Zinc and Linoleic acid and their combination on parameters of block test

Groups	Duration of sniffing self (seconds)	Duration of sniffing non-self (seconds)	Freq. of sniffing self	Freq. of sniffing non-self
Vehicle	10.80 ± 1.393	70.00 ± 7.583	1.600 ± 0.2449	4.000 ± 0.5477
Rotenone	7.000 ± 1.414	1.000 ± 0.633	1.200 ± 0.2000 _a	0.4000 ± 0.2449 _a
Zinc	11.80 ± 2.709*	45.00 ± 5.477*	1.400 ± 0.2449	4.600 ± 0.6782
Linoleic Acid	13.60 ± 2.064*	45.60 ± 4.226*	1.400 ± 0.2449	4.600 ± 0.5099
Zinc + Linoleic Acid	10.20 ± .8602*	49.20 ± 3.839*	1.600 ± 0.2449	6.600 ± 0.9274
Levodopa	8.800 ± .3742 _b	32.40 ± 3.092 _b	1.600 ± 0.2449	2.800 ± 0.3742

Results are expressed as mean ± SEM. *a*, $p \leq 0.0001$ compared to vehicle group; *b*, $p \leq 0.05$ compared to rotenone group; * $p \leq 0.0001$ compared to rotenone group

DISCUSSION

Zinc, linoleic acid and their combination were used in this study to evaluate their protective effect against development of behavioural deficits in rats with parkinsonism induced by rotenone. Precious studies showed that subcutaneous administration of rotenone for a week precipitates both motor and non-motor impairments (Testa et al, 2005) including difficulties in the locomotor activity, decreased rearing behaviour, decreased anxiety and olfactory disturbances. Interestingly, pre-treatment of rats with Zinc or Linoleic acid improved several behavioural parameters. These improvements correlated with the enhancement of antioxidant activity.

Rotenone an alkaloid used often as a pesticide inhibits complex I of the respiratory chain. It is used in animal studies to generate toxin-based rodent models of Parkinson's disease as it increases oxidative stress (Sherer et al., 2007; Tanner, 2011). Rotenone provides a valuable model for studying both mechanisms of oxidative stress and neuroprotection by antioxidant agents because of its mechanism of action involving oxidative stress (Perfeito et al, 2012).

Neuropathologically, rotenone induces the degeneration of the nigrostriatal dopaminergic pathway which is one of the cardinal pathological markers of Parkinson's disease (Tanner, 2011; Testa et al., 2005). As shown in our study, at the behavioural level, rotenone precipitates behavioural alterations including both motor and several non-motor deficits (Inden et al., 2011). We have also shown in a previous study that rotenone induced oxidative stress and histological alterations in the midbrain (Mbiydzennyuy et al., 2018). These alterations have been shown to correlate with the extent of striatal lesions of the rotenone-treated rats (Mulcahy, Walsh, Paucard, Rea, & Dowd, 2011).

Motor performances in all our experimental groups were assessed using the Open Field Test (Stanford, 2007). Remarkably, pretreatment of rats with Zinc protected the animals from the behavioural effects of Rotenone. In fact, rats treated with Zinc showed a significant increase in the frequency of ambulation and the numbers on entries in the elevated plus maze. This enhancement of locomotor activity in the Zinc group could be due to the relatively higher dose of the Zinc (30mg/kg) used in our study corroborating the already observed dose-dependent effect of Zinc on locomotion as assessed by the EPM (A Partyka, Jastrzebska-Wiesek, & Nowak, 2010). Regarding Linoleic acid, no significant effect on motor activity was observed. This result corroborates with the studies of Bourre et al. (1989) in which diet rich in alpha- linoleic acid improved radial maze learning performance without affecting motor activity. Rearing is an important item of the behavioural repertoire in rodents used to explore the environment (Fleming et al., 2004). In the open field test, rearing as a vertical movement is more sensitive to nigral dopaminergic degeneration relative to horizontal movements (Carola et al., 2002). Like its effect on locomotor activity, Zinc prevented the deterioration of the rearing behavior induced by rotenone treatment; similar to Zinc pretreatment, Linoleic acid protected the rearing behavior of rats against the impairment effect of rotenone treatment. In a study on the role of omega-3 fatty acid status on rat adaptation to chronic restraint stress, Linoleic acid-enriched diet was able to abolish the stress-induced reduction in locomotor activity and rearing in the open field test (Calon & Cole, 2007). Zinc has been implicated in the metabolism of Linoleic acid to its other metabolites such as DHA and then to prostaglandins leading to diminished availability of Linoleic acid to influence motor activity (Iso et al., 2002).

Zinc and Linoleic acid exhibited anxiolytic-like effects in rats, as they both increased the percentage of time spent on open arms and the number of entries into the open arms. Although Samardzic et al. (2013) reported an anxiogenic effect of Zinc (30mg/kg), the anti-anxiety effect of Zinc shown in our study is consistent with previous studies (Erreger & Traynelis, 2008; Wlaż et al., 2011). The route of administration could account for these conflicting results. The bioavailability of Zinc has been shown to be variable depending on the route of administration (Bray & Bettger, 1990). Mechanistically, the anxiolytic effect of Zinc has been linked with the inhibition of glutamate NMDA/AMPA receptors and an increase BDNF gene expression in the hippocampus (Brocardo et al., 2007; Szewczyk et al., 2008). Moreover, the reported modulation of the serotonin

neurotransmission by zinc could underlie its effect on anxiety-like behaviour (Anna Partyka et al., 2011; Samardzic et al., 2013). Linoleic acid has been suggested to act as an anxiolytic agent by preventing cellular inflammation as well as improving the antioxidant capacity of cells (Dyall & Michael-Titus, 2008; Ross, 2009; Seki, Tani, & Arita, 2009). Omega 3 PUFAs have been shown to prevent the development of stress-related disorders such as anxiety and depression by protecting glutaminergic neurotransmission in stress induced damage (Denis, Potier, Vancassel, Heberden, & Laviolle, 2013). In a study to investigate the beneficial effect of fish oil on anxiety, depression and cognitive behavior in olfactory bulbectomised rats, Pudell and colleagues suggested that the anti-anxiety effect of omega-3 PUFAs could be due to its ability to increase serotonin levels in the brain (Pudell et al., 2014).

Impaired odour detection, differentiation, and identification has been correlated positively with an increased risk of developing PD, suggesting that behavioural testing of olfaction can facilitate an earlier detection of the disease (Doty et al., 1984; Henderson, Lu, Wang, Cartwright, & Halliday, 2003). These changes are not responsive to dopaminergic therapies (Tillerson et al., 2006). The rotenone group showed significant decrease in sniffing and exploration times. Zinc and Linoleic acid significantly increased the frequency and duration of sniffing the novel scent indicating that they improved the olfactory senses in comparison to the rotenone group in which this was highly impaired. Zinc is believed to play an important role in the regeneration of receptor cells not only on taste buds, but also on the olfactory epithelium (Slotnick, Sanguino, Husband, Marquino, & Silberberg, 2007). Therefore, Zinc deficiency is thought to produce olfactory disorder (Duncan-Lewis, Lukman, & Banks, 2011; Jafek, Linschoten, & Murrow, 2004) although details of the mechanism by which it does so are still unclear. The mechanism by which Linoleic acid improves olfactory function is not also clear but has been linked to its ability to its antioxidant role in the olfactory epithelium as well as neural development (Karr, Alexander, & Winningham, 2011).

The data obtained from biochemical and histological assessments in a previous study with the same experimental protocol also revealed that systemic administration of rotenone (2.5 mg/kg doses, s.c.) in rats for seven days produced increased midbrain lipid peroxidation and impaired antioxidant status, accompanied by histological changes. Pretreatment with zinc, linoleic acid and their combination prevented the increase in MDA levels and decrease in brain antioxidant status induced by rotenone treatment. Histological changes such as cell death and reduction in neuron size induced by rotenone was also prevented by pre-treatment with zinc, linoleic acid and their combination (Mbiydzennyuy et al., 2018).

In conclusion, results of the present study show that repeated systemic administration of rotenone (2.5 mg/kg doses, s.c.) in rats produced functional impairment in the form of bradykinesia in the open-field test and anxiety-related behaviors in the elevated plus maze. The study showed that Zinc significantly increased the locomotor and exploratory activity of rats compared to Linoleic acid and their

combination against rotenone treatment as shown by the open field test. Zinc also showed significant anxiolytic effect compared to Linoleic acid or their combination. Zinc, Linoleic acid and their combination significantly improved olfactory acuity and discrimination as shown by the duration and sniffing of vanilla and non-self bedding in the novel scent and block tests respectively. Zinc and Linoleic acid show prospects of slowing the onset of behavioural deficits that occur early at the outset of Parkinson's disease. However further studies are needed to explore the possible mechanisms involved in these behavioural effects

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