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Aromatic spice Nutmeg attenuates memory deficits in Rotenone model of Parkinson's disease

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ABSTRACT

PD is a multifactorial neurodegenerative disorder with features such as tremor, rigidity, bradykinesia, postural instability, and dementia. Neuropathologically, selective loss or death of dopaminergic neurons is the hallmark of PD. In PD, elevated oxidative stress, mitochondrial dysfunction and neuroinflammation were reported. Rotenone (an isoflavone obtained from Fabaceae associated vegetation such as the jicama vine plant) induces oxidative stress, mitochondrial dysfunction, inflammation and apoptosis in cell line and animal models. It was useful in the evaluation of neuroprotective properties on cell line and animal models of PD. Drugs with anti-oxidant potential helped to control the cellular stress, free radical formation, neurotransmitter level in PD animal models. Nutmeg, an aromatic spice exhibited memory enhancing, anti-oxidant, anticonvulsant properties. It is an excellent body detoxifier and stimulator of the brain due to the presence of pharmacologically active compounds such as eugenol, isoelemicin, isoeugenol, methoxyeugenol, myristic acid, myristicin, saponins and lignin. Macelignan (a compound present in nutmeg) having the low molecular weight and hydrophobic nature could pass beyond the blood-brain barrier. In this study, we explored the cognitive profile of rotenone-induced model of PD treated with MFSE extract (MFSE) by behavioural tests (Morris water maze test, T-maze test and Elevated plus maze Test). Rotenone was injected to male Wistar albino rats by intraperitoneal route (2.5mg/kg daily) for 30days. MFSE treated rats showed significant improvement in cognition in rotenone-induced PD model. It might be due to its neuroprotective and anti-cholinesterase properties.

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INTRODUCTION

PD is a persistent neurodegenerative ailment mainly affecting the locomotory behaviour of the aged population. Symptoms of PD are resting tremor, rigidity, inability to initiate movements, bradykinesia, poor balance in posture and dementia. Neuropathologically, PD is considered with discriminating loss of dopaminergic neurons in the nigrostriatal pathway (SN) and accumulation of insoluble proteins called α-synuclein inside the neurons, developing intra-cytoplasmic structures called as Lewy bodies (Parashar and Udayabanu, 2017). Accumulat-

ing proof suggests that elevated oxidative stress, mitochondrial dysfunction and neuro-inflammation interrelate among, and eventually produce the neuronal death (Schapira and Jenner, 2011). Although the aetiology of PD is not known, it can be either inherited (~10 %) or sporadic (~ 90%) which occurred due to interaction of individual genetic susceptibility with environmental exposure (Sherer *et al.*, 2003). Mitochondrial complex I inhibitor pesticides like rotenone induce discerning destruction of DA neurons and extensively used for experimental PD model (Cicchetti *et al.*, 2009; McDowell and Chesselet, 2012). Rotenone (an isoflavone obtained from Fabaceae associated vegetation such as the Jicama vine plant) (Bové *et al.*, 2005) induces oxidative stress, mitochondrial dysfunction, inflammation and apoptosis in cell line and animal models (Gobi *et al.*, 2018; Dhanalakshmi *et al.*, 2016).

From ancient days, aromatic spices have been included in the food to improve the flavour and taste. They are considered as a valuable, safe and naturally available source of medicine for treating various diseases because of the numerous pharmacologically active constituents. The seeds of the Nutmeg (*Myristica fragrans*) Houtt belonging to Myristicaceae Family are extensively utilised spice due to their typical aroma and taste (Mishra *et al.*, 2018). It is used to flavour the food substances such as baked items, puddings, sweets, sausages, meats, saucers, vegetables and beverages (Panayotopoulos and Chisholm, 1970). It was used in Ayurveda, Unani, Chinese and folklore medicine (Neeraja and Margaret, 2016) to treat gastrointestinal dysfunction, rheumatism, obesity, diarrhoea and sleeping disorders (Vangoori *et al.*, 2018). It exhibited antifungal, spasmolytic, carminative, hepatoprotective, antiviral, anti-carcinogenic and anti-oxidant properties. It is an excellent body detoxifier and stimulator of the brain due to the presence of pharmacologically active compounds such as eugenol, isoelemicin, isoeugenol, methoxyeugenol, myristic acid, myristicin, saponins and lignin (Vangoori *et al.*, 2018).

Jissa *et al.* (2014) indicated that the oral administration of nutmeg attenuated memory deficits and decreased the acetylcholinesterase activity in rats. Nutmeg exhibited anticonvulsant activity against maximum electroshock, pentylenetetrazol and lithium sulphate-pilocarpine nitrate treatment in rats (Sonavane *et al.*, 2002). Recent research revealed that chronic dietary intake of Nutmeg seed enhanced beneficial, productive effect in the rat puppy's brain mitochondria, which is related with an increasing DA receptor (Veronica *et al.*, 2018). Myristica fragrans kernel and seed extract have anti-oxidant, anti-inflammatory, and anti-apoptotic properties against paracetamol and high-fat diet-induced experimental models of hepatotoxicity (Dkhil *et al.*, 2019; Sethi and Dahiya, 2018). In this study, therefore, we studied the cognitive behaviour of rotenone administered PD model with Myristica fragrans seed extract (MFSE).

MATERIALS AND METHODS

Chemicals

Rotenone was procured from Sigma Chemical Company, Bangalore, India.

Animals

Male Albino Wistar rats (225–250 g) were obtained, lodged in Polypropylene cages Central Animal House, Government Theni Medical College, Theni under normal conditions 12 hrs light / dark cycle and 60% humidity, without any restrictions to a standard pellet diet and water ad libitum. Animals were adapted for a week before initiating the experimental protocol. The experimental procedures were permitted by the Animal Ethics Committee of the Institute (Approval no: IAEC/02/2017).

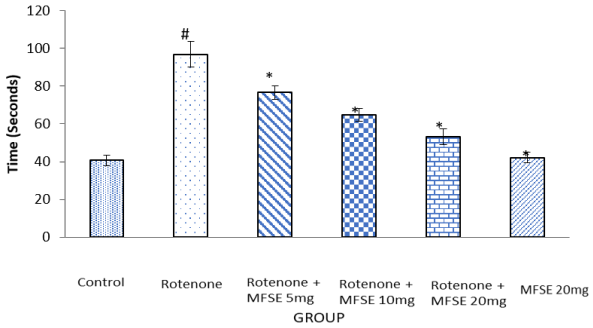

Figure 1: Effect of MFSE on T- maze test of experimental animals

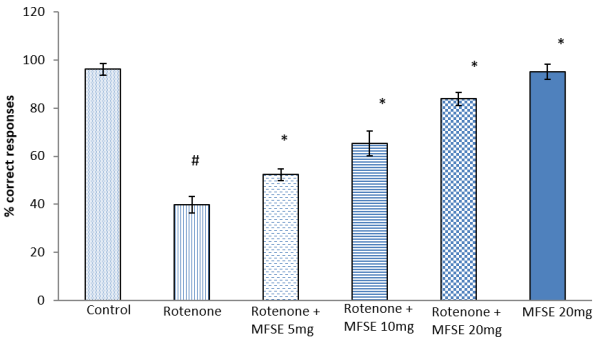

Figure 2: Evaluation of memory by Morris water maze test

Experimental design

In the study, 36 rats were randomly grouped (n = 6):

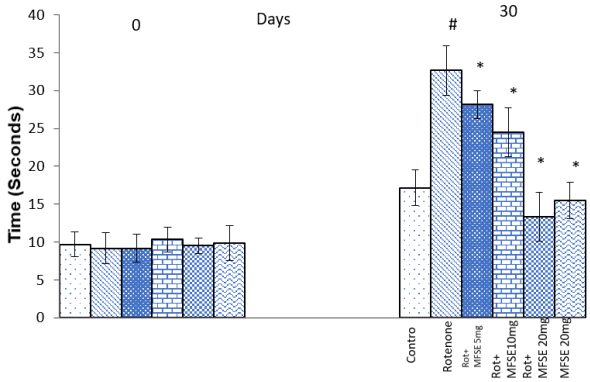

Figure 3: MFSE treated groups displayed gradual decline in TL in EPM test

control rats (0.5 ml of sunflower oil i.p. for 30 days), rotenone only (2.5 mg/kg/day i.p in sunflower oil for 30 days), received rotenone (as group II) (Morais *et al.*, 2012) and a low dose of MFSE(5 mg/kg in saline was treated p.o. after one hour of rotenone treatment and continued up to 30 days) treated, rotenone and intermediate dosage of MFSE (10 mg/kg in saline p.o. for 30 days) treated, rotenone and a high dose of MFSE (20 mg/kg in saline p.o. for 30 days) treated and MFSE (20 mg/kg/day p.o. for 30 days) alone treated. T-maze test, Morris water maze test and Elevated plus Maze Test were performed.

Group I: Control (0.5 ml of sunflower oil i.p. for 30 days)

Group II: Received Rotenone **(**2.5 mg/kg/day i.p in sunflower oil for 30 days)

Group III: As group II + (After 1-hour ROT) MFSE (5mg/kg) p.o. for 30 days

Group IV: Received.as group II + (After 1 hour ROT) MFSE (10mg/kg) p.o. for 30 days

Group V: Received as.group II + (After 1 hour ROT) MFSE (20mg/kg) p.o. for 30 days

Group VI: MFSE (20mg/kg) p.o for 30 days

Preparation of nutmeg extract

MFSE extract was prepared, as mentioned by Ghorbaniana *et al.* (2019).

T-maze test

This test was performed as mentioned (Yang *et al.*, 2017). Shortly, the animals training were conducted in for two consecutive days (23 and 24). T-maze, rewards (food-choco chips Kelloggs) were kept at the end of one arm. Detection of food associates working memory. Each practising session comprised of 9 rounds, each round consists of two parts – forced run, optional run. In the forced run, one of the arms was shut with a sliding door; another arm was kept open with food at its end. After 30 sec, the choice run was allowed; both arms were free, reward (foods) were placed at the ends of two arms. The appropriate response was choosing the newly opened arm, which was shut at the forced run. On day 28, tests were taken, and its observations were noted. The analysis comprised of 3 run trials; animals were subjected to 2 optional runs following 1 forced run. After the trial sessions on 23 and 24^{th} day, animals were given access to food only for 1 hour, i.e., partial restriction to diet. On the experimental (25^{th} day) day, access to food was restricted before the test. After the animals completed their behavioural tests, there is no restriction to food ad libitum.

Morris water maze test

This test was carried out for evaluating the environmental knowledge and memory evaluation. In the test room, within a circular tub (71 inches diam $\times$ 24 inches), rats were permitted to swim to a stage. The tub was employed with tap water ($82\pm20\,^{\circ}$F) to a depth of 16inches. A round stage 4inches in diameter was positioned inferior to 2cm of water level, and water was made opaque by adding white talcum powder. On the 27^{th} day, animals were trained in session having four trials. Beginning positions were set dissimilar in all four trials. The time taken to locate the stage was noted up to a max 2 min. During the experiment, the stage was stationary and kept in position. The duration to board the stage was carefully documented. After 24 h (i.e., 28th day), rats were randomly placed separately over the brim of the pool and tested. Duration for getting into the hidden stage on 28^{th} day was taken into account as retention latency (Su *et al.*, 2010).

Elevated Plus-Maze (EPM) Test

The cognitive skill of the animal was analysed by this EPM method. Concisely, the equipment contained dual closed and double-exposed arms with a dimension of 50x 10x 40cm with an open roof; it was kept high above the ground level (50cm). The duration taken to travel from exposed arm to closed arm was noted as transfer latency (TL). Initially, rats were gently introduced into the open arm, and TL was taken into account. In case, the experimental animal was unable to enter into the closed part within 90 sec, we gently forced the animal to enter into it, and 1.5 min was noted for TL. As soon as the rats enter the closed arm, rats were permitted to discover the maze for 20sec; animals returned to their cages. On day 0 and 30, the TL was documented (Itoh *et al.*, 1991).

RESULTS

Figure 1 shows that treatment with MFSE remarkably ameliorated rotenone intoxicated cognitive dysfunctioning in T-maze.test. Each bar represents results as mean ± SD; n=6/group #P<0.001 compared with normal group and *P<0.001 compared with rotenone group using one-way analysis of variance. In our study, diminished functional memory was characterised in rotenone administered group by drastic declined precise responses, whereas MFSE enhanced the scores of right answers. MFSE improved the working memory by detecting the food within short period of time.

Effect of MFSE on memory evaluation in the Morris water maze test

On comparing with the control group, spatial memory dysfunction was found to be notable in rotenone alone group. From dosage of MFSE 5- 20mg, gradual improvement of spatial memory of rats was exhibited in the MFSE + Rot group (Figure 2). Each bar indicates mean±SD n=6 #P<0.001 compared withnormal group and *P<0.001 compared with rotenone group by one-way analysis of variance. MFSE 20mg group showed a similar behavioural profile of control groups.

MFSE attenuates the cognitive skill in the EPM test

With respect to the control group, TL was remarkably increased in the rotenone intoxicated group. On relating with rotenone intoxicated group, rot + MFSE group exhibited a sharp decline in TL. Each bar shows mean±SD, n=6, #P<0.001 compared with normal group and *P<0.001 compared with rotenone group using one-way analysis of variance (Figure 3).

DISCUSSION

Environmental exposure to pesticide such as rotenone might lead to neurodegenerative disorders like Alzheimer's disease, PD, Huntington's disease, Leber optic neuropathy. Precautions and safety measures must be followed to reduce the risk (Zhang *et al.*, 2006). Chronic administration of rotenone had been proved to produce cognitive and learning deficits (Kaur *et al.*, 2011). Deterioration of cognitive skills was witnessed in PD. It was reported that the cholinergic neurons were associated with CNS activities, including sleep and cognition (Shinomol and Muralidhara, 2011). Cholinesterase inhibitors were recommended for PD patients as they are having significant cholinergic deficits (van Laar *et al.*, 2011).

Intra-peritoneal rotenone injection resulted in cognitive impairment and reduced memory index. The striatum comprises of a population of cholinergic interneurons, and there are indications that cholinergic neurotransmission plays a role in striatal function (Müller and Bohnen, 2013). This could have contributed to the cognitive decline so observed. Drugs with anti-oxidant potential helped to control the cellular stress, free radical formation, neurotransmitter level in PD animal models. Hence, they are extensively preferred as promising anti-neurodegenerative agents. Cholinesterase inhibitors were stated to have a beneficial and therapeutic effect on cognitive disabilities (Kamal *et al.*, 2015).

Several plants derived compounds such as brown rice (Chompoopong *et al.*, 2016), cinnamaldehyde (Mehraein *et al.*, 2018), pepper (Ogunruku *et al.*, 2019) and drugs such as Ceftriaxone (Ruzza *et al.*, 2014; Ho *et al.*, 2014), piracetam (Verma *et al.*, 2015) were proved to be effective in treating dementia in experimental PD. Marine-derived natural products such as omega-3- fatty acid, inosine, etc., were currently under clinical trials against PD (Huang *et al.*, 2019). Moreover, Ho *et al.* (2011) revealed that D- cycloserine was advantageous in the management of biochemical, biological detrimental changes in experimental PD, including cognitive disorders, motor dysfunction.

MFSE were illustrated to enhance the cognitive and intellectual abilities in rats (Jissa *et al.*, 2014). MF extract was capable of reducing acetylcholinesterase activity which is more beneficial in the treatment of Alzheimer's disease (Singh *et al.*, 2020). Nutmeg oil was evidenced to be effective in the management of epilepsy (Ayaz *et al.*, 2017). Macelignan (a compound present in nutmeg) having a low molecular weight and hydrophobic nature could pass beyond the blood-brain barrier (Wu *et al.*, 2016). Allylguaiacol a compound present in numerous spices like cloves, cinnamon, basil, and nutmeg offers neuroprotective effect due to its anti-oxidant property, anti-apoptotic property and control of Transcription factor p65. Allylguaiacol could be fruitful in controlling the memory deficits in Alzheimer's, PD also other ailments (Lim *et al.*, 2018).

In a recent study, it was proved that MFSE was effective in controlling memory deficits in Alzheimer's disease (Singh *et al.*, 2020). This research study indicated that MFSE treated groups exhibited significant improvement in suitable reactions during T- maze test, a substantial reduction in duration to board the hidden stage in the MWM test and TL in the EPM test.

CONCLUSION

This study demonstrates that MFSE was effective against memory deficits in rotenone-induced PD model. It could be as a result of its promising cholinesterase inhibition, anti-oxidant, anti-carcinogenic, anti-epileptic properties. Still, further detailed research is in need to explore the underlying mechanism and compound responsible for it.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Ethical clearance

The Experimental Protocols were approved by the Institutional Animal Ethical Committee (Reg. No. 1112/2007/CPCSEA, Proposal No. 02/2017-GTMC).

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