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Evaluation of myristica dactyloides seeds extract on rotenone induced dopamine depletion, dopaminergic neurodegeneration and oxidative imbalance in a rat model of PD

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Abstract:

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss and oxidative stress. The present study investigated the neuroprotective effect of Myristica dactyloides seed extract (MDSE) against rotenone-induced Parkinsonism in rats. Male Wistar rats were divided into six groups and treated with rotenone (2.5 mg/kg, i.p.) alone or in combination with MDSE (5, 10, and 20 mg/kg, p.o.) for 30 days. Neurochemical parameters including dopamine (DA) and its metabolites, oxidative stress markers such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were evaluated. Rotenone administration significantly depleted striatal dopamine levels, increased lipid peroxidation, and reduced antioxidant enzyme activities. Furthermore, rotenone reduced the expression of dopaminergic markers including tyrosine hydroxylase (TH), dopamine transporter (DAT), and vesicular monoamine transporter-2 (VMAT-2). Co-administration of MDSE significantly attenuated rotenone-induced neurochemical and oxidative alterations in a dose-dependent manner. The highest dose of MDSE (20 mg/kg) markedly restored antioxidant status and dopaminergic function. These findings demonstrate that MDSE possesses significant neuroprotective activity against rotenone-induced Parkinsonian neurodegeneration, likely through antioxidant and dopaminergic protective mechanisms.

Keywords: Parkinson's disease, Myristica dactyloides, Rotenone, Dopamine, Oxidative Stress

Introduction:

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to depletion of dopamine in the striatum and impairment of motor functions. Oxidative stress, mitochondrial dysfunction, neuroinflammation, and α-synuclein aggregation are considered major contributors to PD pathogenesis (Hoglinger et al., 2003). Rotenone, a mitochondrial complex-I inhibitor, is widely used to induce experimental Parkinsonism because it

reproduces many pathological and behavioral features of human PD, including dopaminergic neuronal degeneration and oxidative damage (Sherer et al., 2003).

Oxidative stress plays a central role in neuronal degeneration by promoting lipid peroxidation, protein oxidation, and DNA damage. The brain is particularly vulnerable due to its high oxygen consumption, abundant lipid content, and relatively low antioxidant defense mechanisms (Barnham et al., 2004). Natural products possessing antioxidant and neuroprotective properties have attracted considerable interest as potential therapeutic agents for neurodegenerative disorders.

Myristica dactyloides, a medicinal plant belonging to the family Myristicaceae, contains several bioactive phytoconstituents with reported antioxidant properties. However, its neuroprotective potential against Parkinsonian neurodegeneration remains largely unexplored. Therefore, the present study was undertaken to evaluate the protective effect of Myristica dactyloides seed extract (MDSE) against rotenone-induced dopamine depletion, oxidative stress, and dopaminergic neurodegeneration in rats.

Materials and method

Procurement of plant and preparation of extract:

Myristica dactyloides were procured from Krishna Departmental store, Theni, Tamilnadu authenticated by Dr. Sankaraj M.D (Siddha) then dried in shadow. Using a mechanical grinder, 300 gm of nutmeg seeds were powdered. Then, absolute ethanol (500 ml) was added; the powder was mixed and shaken for 72 h and the supernatant solvent was removed by using a rotary evaporator (in a vacuum, temperature under 45°C). For evaporating the solvent entirely, the extract was collected in a plastic bottle and then stored at −80°C to prevent oxidative damage (Ghorbanian et al 2019).

Animals

Male Albino Wistar rats (225–250 g) kept at Polypropylene cages, Central Animal House, Government Theni Medical College, Theni below preferred conditions 12 hrs light / dark cycle and 60% humidity fed with standard pellet diet and water ad libitum. The experimental protocols met with the National Guidelines on the right care and use of Animals in Laboratory Research and approved by the Animal Ethics Committee of the Institute (Approval no : IAEC/02/2017). Before initiation of the treatment schedule, the animals were practised for a week to attain its maximum performance in behavioural tests.

Experimental design

In the experiment, animals were randomly grouped (n = 6): control rats group I (0.5 ml of sunflower oil i.p. for 30 days) , rotenone treated (2.5 mg/kg/day i.p in sunflower oil for 30 days) only (as group II) (Morais et al., 2012), low dose of MDSE (5 mg/kg in saline) treated p.o. after 1 h of rotenone treatment and continued for 30 days (as group III), intermediate dosage of MDSE (10 mg/kg in saline) p.o. after 1 h of rotenone treatment and continued for 30 days rotenone (group IV) and high dose of MDSE (20 mg/kg in saline) treated p.o. after 1 h of rotenone treatment and continued for 30 days (group V) and MDSE (20 mg/kg/day p.o. for 30 days) alone treated (group VI). Open-field test, rotarod, traversing the narrow beam test and hang test were performed after 32 h of the final day of experiment. Biochemical (TBARS, SOD, Catalase, GPx and GSH) and neurochemical (dopamine and its metabolites) parameters were analysed. Another set of experiment using 36 rats were repeated for behaviour studies (Modified forced swim, Sucrose preference and

Elevated plus maze test) and to analyse the proteinstudies of dopaminergic and inflammatory markers.

Results and discussion

Effect of MDSE on rotenone induced dopamine depletion, dopaminergic neurodegeneration and oxidative imbalance in a rat model of PD

Animal models are mainly used to study the etiology, pathology and molecular mechanism of PD. It has replicated almost all of the hallmarks of PD and useful for testing new neuroprotective or neuro-restorative pharmacological extracts or compounds. The experimental model displays physiological symptoms (behavioural deficts) similar to human PD and used to understand molecular changes and pathophysiology in patients with PD. Of the neurotoxic models, compounds that produce both irreversible (MPTP, 6-OHDA, paraquat, rotenone) and reversible (reserpine) effects have been used effectively; however recent experiments have much focused on irreversible toxins to produce PD related pathology and symptomatology (Hoglinger et al., 2003). Chronic administration of rotenone induces the inhibition of the mitochondrial ETC in rat brain. In rodents, i.p. injections have been reported to elicit behavioral and neurochemical deficits (Alam et al., 2004), α-synuclein aggregation and Lewy body formation (Sherer et al., 2003). A significant reduction in striatal DA content (Bazzu et al., 2011) and its catabolites like DOPAC and HVA (Serra et al., 2002) are the one of the important hallmark in PD (Rocchitta et al., 2005). Catecholamine biochemical and transport dynamics have been extensively studied in both in vivo and in vitro experiments of neurological disorders (Chiou et al., 2007).

Rotenone created an environment of significant oxidative and nitrosative stress, which leads to α-synuclein aggregation and Lewy body formation. Generally, dopaminergic impairment is assessed by measuring DA level. Therefore, the present study was aimed to evaluate dose dependent effect of MDSE against rotenone induced neurochemical deficits dopaminergic degeneration and oxidative stress in rat model of PD.

Effect of MDSE on rotenone induced DA and its metabolites depletion in ST

The level of DA and its metabolites in ST of control and experimental rats were shown in Fig.19 A and B. From the results, it was observed that rotenone administration (2.5 mg/kg, i.p. for 10 days) produced a significant depletion of striatal DA, DOPAC and HVA levels, whereas the rats treated with MDSE alone (20 mg/kg) showed no substantial change as compared to control. Co- treatment with MDSE dose dependently attenuated rotenone induced depletion of striatal DA level in rats and from the result it was observed that 10 mg/kg of MDSE administration showed more significant induction in DA level as compared to 5 mg/kg of MDSE administration. Meanwhile, no significant changes were found between the middle and high dose of MDSE and rotenone treated rats.

Effect of MDSE on rotenone induced levels of MDA and antioxidants in SN

The level of MDA was significantly increased, whereas the activities of SOD, catalase and GPx were significantly decreased in the SN of rotenone treated rats as compared to control rats (Table 3). However, these changes in the level of TBARS and the activities of SOD, catalase and GPx were significantly attenuated in rats co-treated with MDSE (5, 10 and 20 mg/kg. b.w) as compared to rotenone alone treated rats (group II). There is no significant difference in the level of MDA and activities

of SOD, catalase and GPx between MDSE and control animals.

Effect of MDSE in expression of dopaminergic markers on rotenone PD model Administration of rotenone caused an increase in the expression of TH, DAT and VMAT-2 as compared to control rats. Co-administration of MDSE to rotenone treated rats significantly enhanced the expression of dopaminergic markers as compared to rotenone alone treated rats. No significant changes were observed between control and MDSE alone treated rats.

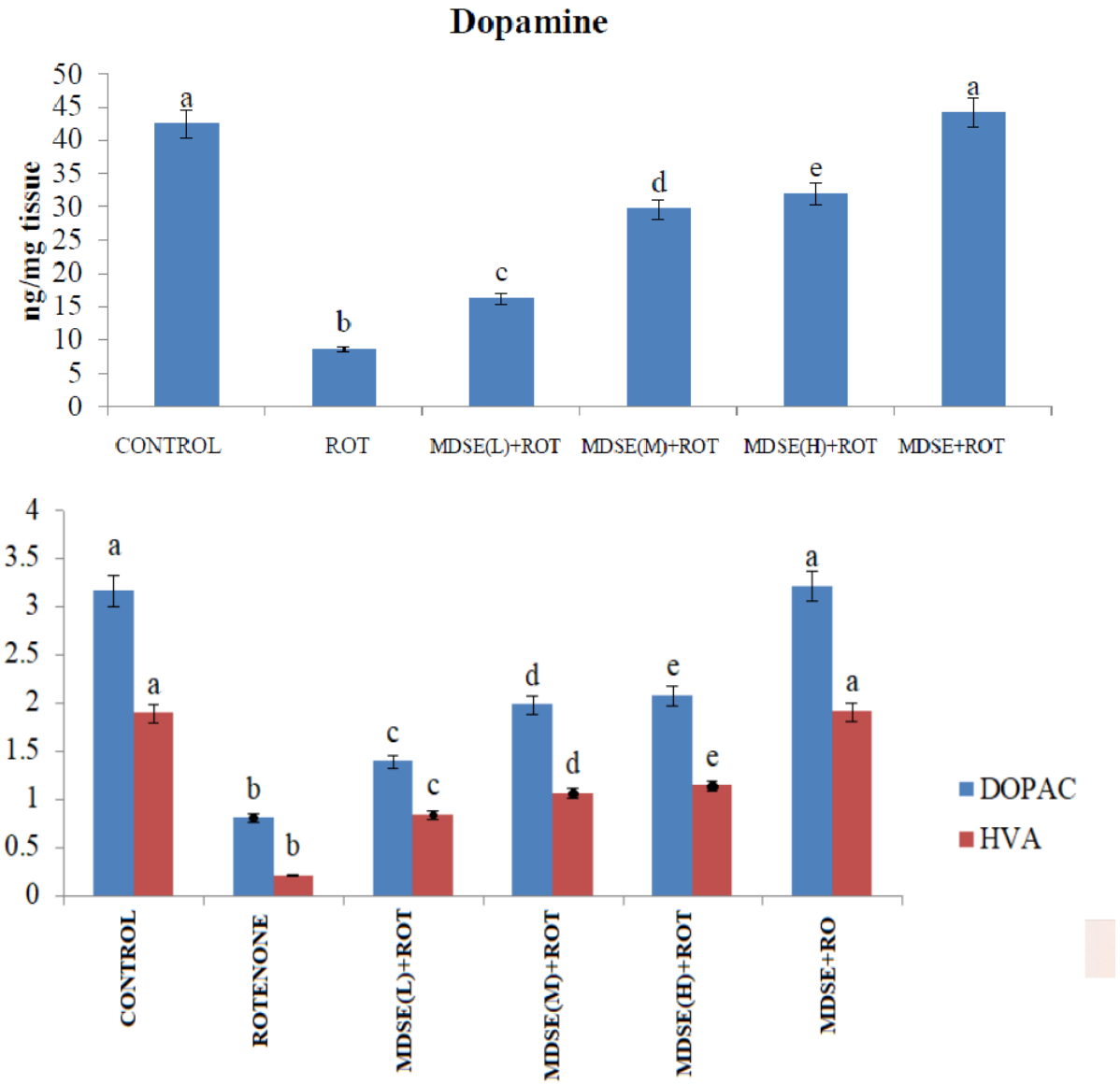
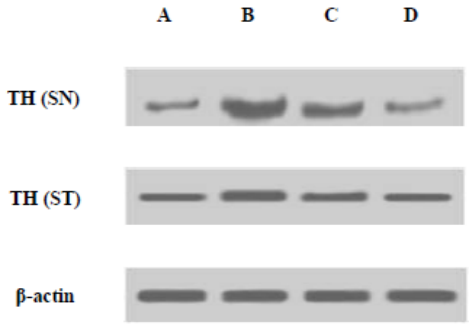


Fig. 1 A and B illustrates the striatal DA and its metabolites level in control and experimental animals. Values are given as mean±SEM for six rats. Values not sharing the same alphabet differ significantly ($p<0.05$).

Table 1: Effect of Myristica dactyloides seed extract (MDSE) on lipid peroxidase (LPO) and first line antioxidant defense in corpus striatum of rotenone intoxicated rats

S. No.	Group	LPO (nmole MDA/mg protein)	SOD (unit/mg protein)	Catalase (unit/mg protein)	GPX (unit/mg protein)
1	Control	2.27 ± 0.43	9.86 ± 0.29	3.42 ± 0.33	5.12 ± 0.37
2	Rotenone	6.61 ± 0.51#	3.71 ± 0.31#	1.71 ± 0.29#	2.10 ± 0.27#
3	Rotenone + MDSE 5 mg	5.25 ± 0.38*	4.25 ± 0.26*	2.22 ± 0.47*	2.90 ± 0.23*
4	Rotenone + MDSE 10 mg	4.56 ± 0.22*	5.80 ± 0.35*	2.62 ± 0.24*	3.62 ± 0.19*
5	Rotenone + MDSE 20 mg	3.47 ± 0.27*	7.46 ± 0.26*	3.13 ± 0.21*	4.47 ± 0.18*
6	MDSE 20 mg	2.15 ± 0.61	9.93 ± 0.32	3.68 ± 0.42	5.24 ± 0.34

The effect of MDSE on rotenone induced oxidative stress in rat model of PD. Rotenone treatment significantly increased the levels of TBARS and diminished the activities of SOD, catalase and GPx as compared with control animals, while MDSE treatment significantly attenuated rotenone-induced oxidative stress. Values are given as mean ± SD (n=6), values not sharing common superscript are significant with each other, P <0.05, ANOVA followed by DMRT. A. Enzyme concentration required for 50% inhibition of NBT reduction in 1 minute. B. Micromoles of hydrogen peroxide consumed per minute. C. Micrograms of glutathione consumed per minute.



A. Control; B. Rotenone; C. Rotenone+*MDSE*; D. *MDSE*

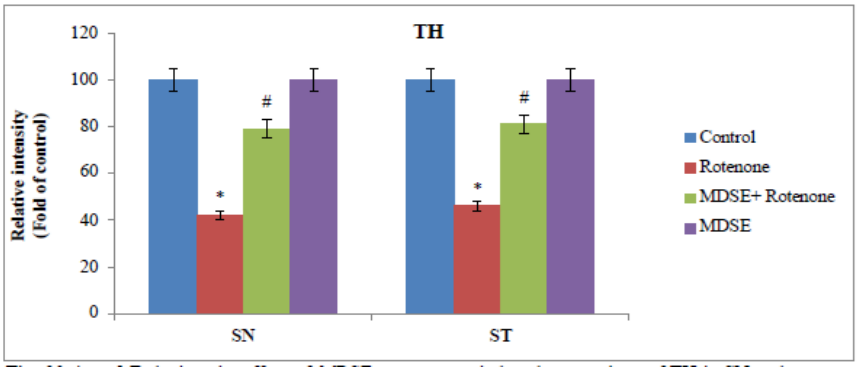


Fig. 2 A and B depicts the effect of MDSE on rotenone induced expressions of TH in SN and ST of control and experimental rats. Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as arbitrary units and data are shown as mean±SEM. *p<0.05 compared to the control rats, #p<0.05 compared to the rotdenone treated rats.

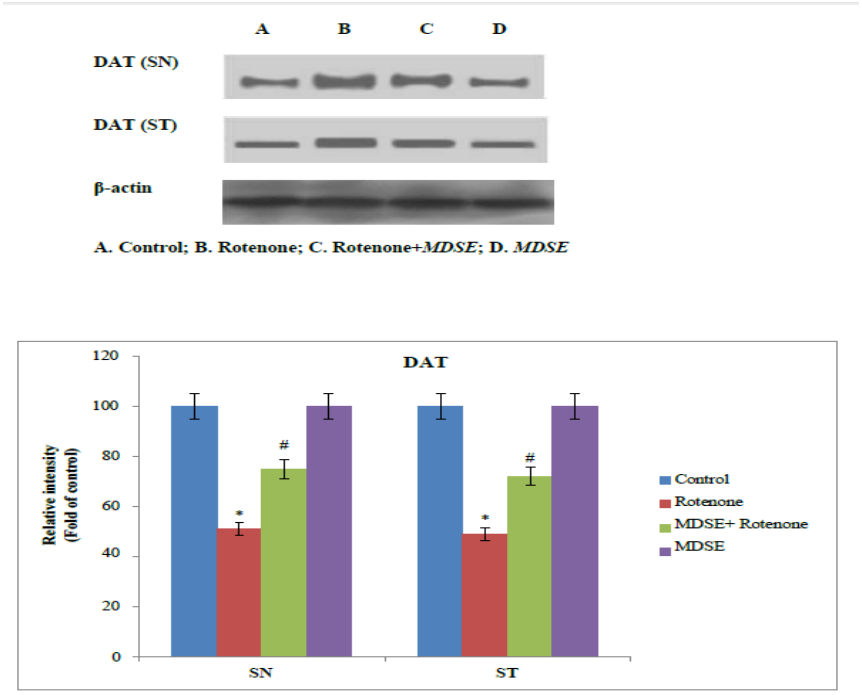


Fig. 3 A and B depicts the effect of MDSE on rotenone induced expressions of DAT in SN and ST of control and experimental rats. Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as arbitrary units and data are shown as mean±SEM. *p<0.05 compared to the control rats, #p<0.05 compared to the rotenone treated rats.

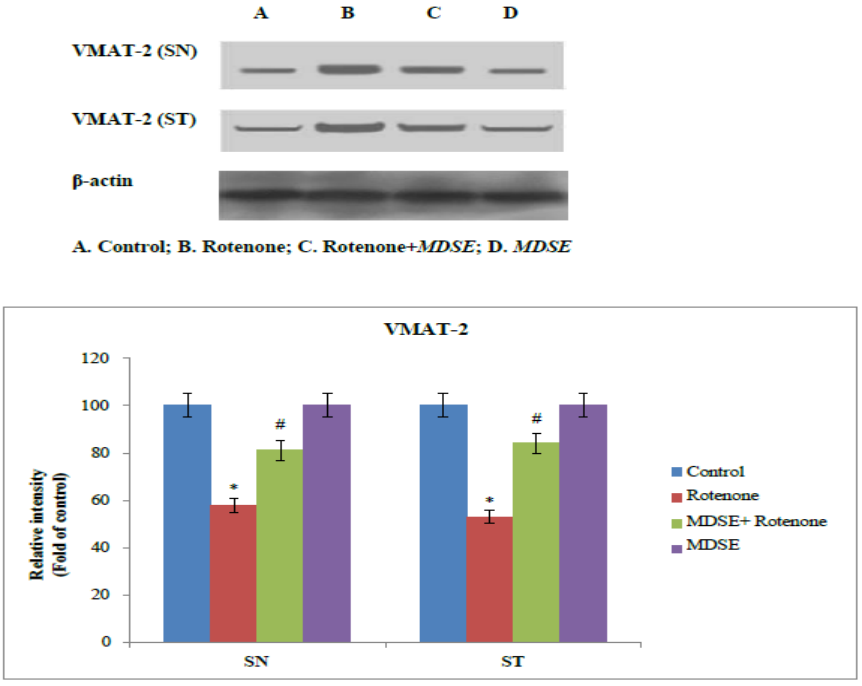


Fig. 4 A and B depicts the effect of MDSE on rotenone induced expressions of VMAT-2 in SN and ST of control and experimental rats. Immunoblot data are quantified by using β-actin as an internal control and the values are expressed as arbitrary units and data are shown as mean±SEM. *p<0.05 compared to the control rats, #p<0.05 compared to the rotenone treated rats.

In our experiment, i.p. administration of rotenone exerted its neurotoxicity by diminishing the level of striatal DA and enhancing the level of TBARS, matched exactly with previous experiment (Zhang et al., 2014). Injection of rotenone at low doses causes DA neurodegeneration in the nigro-striatal pathway (Sherer et al., 2003). Striatal DA loss is the pathological hallmark of PD. The ST possess group of dopaminergic neurons, that is thought to act as a local source of DA and this neurotransmitter plays important role for balance, control of movements and walking (Wachter et al., 2010). The decreased levels of DA cause the neurons of the ST to fire uncontrollably, inhibiting the PD patient to direct motor function. In the present study, rotenone caused a significant decline in levels of striatal DA and its metabolites. If, DA depleted below low striatal levels around 40-60%, then the first motor symptoms of PD occurs (Obeso et al., 2000). Drugs capable to ameliorate rotenone induced DA depletion are deliberated to be neuroprotective. In this context, the co-treatment of MDSE markedly enhanced the level of DA in the ST of rotenone treated rats and proved its neuroprotective function. Brain is vulnerable to oxidative stress because (i) high metabolic activity and utility of more oxygen, (ii) auto-oxidation of DA and its metabolites and (iii) presence of enhanced iron levels in the SN leading to the reduction of H2O2 to produce the highly reactive hydroxyl radical, (.OH) (Fenton chemistry) (Barnham et al., 2004). Post mortem analysis of brains from PD patients confirmed the enhanced level of oxidative stress in the SN marked by elevated iron concentrations (Good et al., 1992), enhanced lipid peroxidation (Dexter et al., 1994) and oxidation of DNA and protein (Alam et al., 1997). Lipid peroxidation is the process of oxidative destruction of PUFA and its occurrence in membranes causes impaired structural integrity and membrane function, inactivation of numerous membrane bound enzymes and decreased fluidity Halliwell and Gutteridge, 1985.

The extent of lipid peroxidation processes was measured by quantifying the level of TBARS, collective products of lipid peroxidation. Bashkatova et al., (2004) showed increased NO and TBARS level in the ST and cortex following the chronic rotenone treatment, which supports with our study. Elevated synthesis of ROS during NDDs is a sign of the oxidative stress and leads to a rapid consumption of endogenous scavenging antioxidants. Rotenone administered PD rats enhanced the level of TBARS and reduced the levels and activities of enzymatic antioxidants which are in consistent with previous studies (Bashkatova et al., 2004; Sharma and Nehru, 2015). Previous studies demonstrated the decreased GSH level and SOD activity (Sherer et al., 2002) and enhanced levels of MDA (Xiong et al., 2009) in rotenone model of PD. SOD is ubiquitous enzymatic antioxidant, present in most tissues and being one of the key antioxidants in the CNS together with catalase and GPx (Evans, 1993). In the present study, diminished activity of SOD in the rotenone treated rats indicated inactivation of SOD by ROS (Wei and Meng, 2010) and leads to the scenario of increased superoxide radical production. Enhanced superoxide radicals spontaneously dismutated to form H2O2. Elevated levels of H2O2 could lead to depletion of their metabolising enzymatic antioxidants such as catalase and GPx. MDSE offered neuro-protective properties by diminishing the levels of lipid peroxidation products and enhancing the antioxidants.

Generally, dopaminergic impairment is assessed by measuring DA levels and immunoreactivity/protein expression of dopaminergic neuron markers such as TH

and DAT. Rotenone administration induced reduction of TH, DAT and VMAT2 expression in both ST and SN which are the important hallmarks of the neurodegenerative changes of dopaminergic neurons in PD. These proteins are helpful in the production and degradation, uptake and release of dopamine in nerves. TH is the rate-limiting enzyme for DA synthesis and also a key molecule in dopaminergic functions because it transforms tyrosine to L-DOPA, which is then converted to DA (Nakashima et al., 2009). Degeneration of DA neurons concurrently reduces the release of DA neurotransmitter that results in remarkable functional deficits in the basal ganglia. Experimental PD animals shows the decrease in the activity of TH and the dramatic fall in TH has been suggested to be of underlying importance in the pathogenesis of PD (Rausch et al., 1988).

DAT is abundant in DA neurons of the nigrostriatal pathway and is the access zone of several toxins including rotenone, OHDA and MPTP (Dauer and Przedborski, 2003). Although, DAT expression is vital for normal dopamine neurotransmission, it also renders the dopamine neuron susceptible to damage by toxins that can be transported by DAT. Degradation of dopaminergic neurons in PD may lead to decrease in DA storage efficiency due to the decreased DAT population on the degraded dopaminergic neurons (Watanabe et al., 2005).

VMAT2 is responsible for packaging intra neuronal DA into synaptic vesicles in preparation for synaptic release and hence it is a important regulator of cytoplasmic DA levels and dopaminergic function. This raises the possibility that VMAT2 may be essential in dopaminergic neurons to prevent cytoplasmic DA oxidation by sequestering DA into vesicles. VMAT2, necessary for normal cell function serves as a neuroprotective factor by appropriating neurotoxin into vesicles. VMAT2 protects dopaminergic neurons from rotenone induced oxidative stress and neurodegeneration by sequestering neurotoxin into cytoplasmic vesicles and inhibiting its toxic action (German et al., 2000).

Co-administration of MDSE remarkably attenuated rotenone induced neurotoxicity via its neuroprotective effect. To conclude, the present study validates the neuroprotective effect of MDSE against rotenone induced neurotoxicity. The protective effect may be caused by enhancing the levels of striatal DA, nullifying dopaminergic markers deficits and inhibiting the oxidative stress.

Conclusion

The present study demonstrates that Myristica dactyloides seed extract possesses significant neuroprotective activity against rotenone-induced Parkinsonian neurodegeneration in rats. Rotenone administration caused marked depletion of striatal dopamine, increased lipid peroxidation, reduced antioxidant enzyme activities, and impaired expression of dopaminergic markers such as TH, DAT, and VMAT-2. Treatment with MDSE effectively attenuated these pathological alterations in a dose-dependent manner. The extract restored dopamine levels, improved endogenous antioxidant defenses, reduced oxidative stress, and preserved dopaminergic neuronal integrity. The findings suggest that MDSE may serve as a promising natural therapeutic candidate for the management of Parkinson's disease through its antioxidant and dopaminergic neuroprotective mechanisms. Further studies are warranted to isolate the active constituents and elucidate their precise molecular mechanisms.

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