BENEFICIAL EFFECTS OF SESAMOL IN BRAIN OF ROTENONE-INDUCED ANIMAL MODEL OF PARKINSON'S DISEASE

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ABSTRACT

To investigate the beneficial effect of sesamol on neurotransmitters of rotenone-induced animal model of Parkinson's disease. Male Wistar albino rats were subjected to study for 60 days (n=6: I- vehicle control, II- rotenone (3 mg/kg.B.wt intraperitoneal), III- rotenone + sesamol (50 mg/kg.B.wt intraperitoneal), IV- rotenone + sesamol + L-DOPA (10 mg/kg.B.wt oral), V- rotenone + L-DOPA). The brains of the experimental animals were homogenized, centrifuged and subjected to analyze the neurotransmitters such as dopamine, epinephrine, nor epinephrine and serotonin. Rotenone has developed neurochemical alterations in the brain of experimental animals whereas administration of sesamol has reduced the alterations induced by rotenone. The results also prove that sesamol can serve as a beneficial compound in the maintenance of neurotransmitters in Parkinsonian models.

Keywords: *Parkinson's disease, rotenone, sesamol, brain, neurotransmitters*

Introduction

Numerous fields of medicine are directly associated with neuroscience (neurosurgery, neurology and psychiatry) **(Fitz Gerald and Folan Curran, 2002)**. In the nervous system, neurotransmitters communicate messages from neurons to neurons or from neurons to muscles. These molecules are also known as body's chemical messengers. These neurotransmitters are amino acids, small amine molecules or neuropeptides. The synaptic cleft is a slight gap present in between the synapses of a neuron. This synaptic cleft is the location where the communication occurs between neurons. The excitatory, inhibitory or modulatory are the routes in which a neurotransmitter stimuli on neurons. The action potential is the electrical signal generated in the receiving neuron by the excitatory transmitter whereas this action in inhibited by the inhibitory transmitter. The receptors decides whether a transmitter to be an excitatory or inhibitory. Glial cells like astrocytes are also involved in the synthesis of neurotransmitters. They can also release the neurotransmitter to the target cell. **(https://qbi.uq.edu.au/brain/brain-physiology/what-are-neurotransmitters)**

Recent advances are made to study the behavior of brain by neurotransmitters. The neurotransmitter system is depicted in the **figure 1.** Its immediate intercellular communication between cells of the brain and the interaction with the cell surface receptors triggers messages and controls the ion channels. Increased consciousness to understand the neurotransmitters and its interaction with receptors improve the routes to manage neurological diseases **(Paulose *et al.*, 2007).** Parkinson's disease or Parkinsonism is a neurodegenerative condition developed due to genetic mutations and environmental exposures. **Dorsey *et al.*, 2007** established that 2% of population above 65 years are commonly affected by PD and also found to increase two fold by the year 2030.

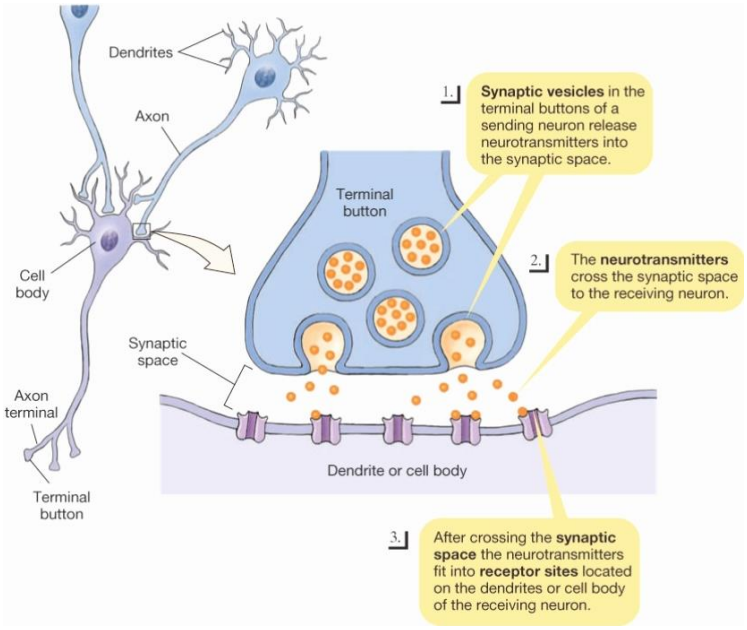

Fig 1: The neurotransmitter system

Source: **http://abdpvtltd.com/neurotransmitter-diagram/neurotransmitter-diagram-best-of-neurotransmitter/**

Catecholamines (dopamine, epinephrine and norepinephrine) are the most significant neurotransmitters in the nervous system **(figure 2)**. The aromatic amino acid (L-tyrosine) is

the source of catecholamines. Dopamine is the chief catecholamine which carries numerous possessions in neuronal and non- neuronal cells. In neuronal cells, dopamine serves as a potent neurotransmitter whereas in non- neuronal cells, it acts as an autocrine and paracrine mediator.

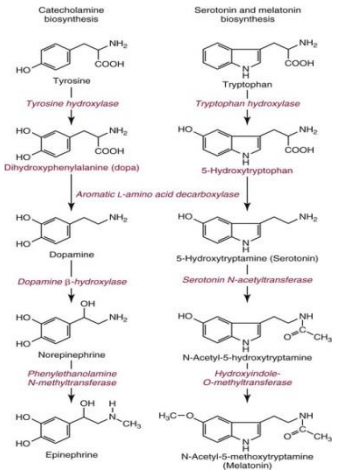

Fig 2: Biosynthesis of neurotransmitters

Source: **https://basicmedicalkey.com/catecholamines-and-serotonin/**

The dopamine system is well organized and recognized **(Figure 3)**. Epinephrine is also called as adrenaline secreted from adrenal glands. It is an excitatory neurotransmitter. It is responsible for the fight and flight response of the body. Increased level of epinephrine is observed in the blood during anger and fear situations. This neurotransmitter also regulates the blood pressure, production of glucose and cardiac rate.

(https://www.kenhub.com/en/library/anatomy/neurotransmitters)

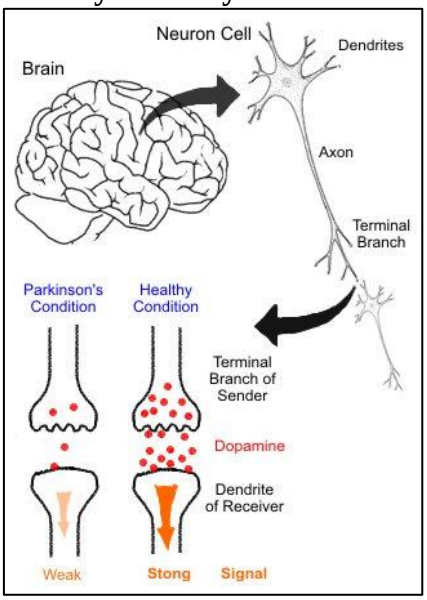

Fig 3: Dopamine in Parkinson's disease

Source: **https://stemedix.com/parkinsons-disease/parkinson2/**

Norepinephrine is synthesized from the adrenal glands and it is an excitatory neurotransmitter. It is also called as noradrenaline. Ulf Von Euler, 1946 was the first person to identify this neurotransmitter. Norepinephrine is much essential to produce epinephrine endogenously. It is known to stimulate the attentiveness of the brain and also handles various processes of the body. It is concerned in the disorders like anxiety and insufficient sleep.

(https://www.kenhub.com/en/library/anatomy/neurotransmitters)

They also revealed that reduction of norepinephrine leads to all the symptoms of PD and the supply of noradrenergic agents in the dopaminergic treatment can diminish the symptoms **(Delaville *et al*., 2011).** The serotonin plays an important role in the activation of projection systems in the central nervous system. The outcome of the serotonin mainly depends on the expression of receptors. There are seven types of serotonin receptors which are excitatory and inhibitory. The serotonergic neurons are established in the nuclei of raphe which is classified into descendent and ascendent serotonergic systems. The descendent serotonergic system project to the caudal parts present in the central nervous system (spinal cord, cerebellum and brain stem). The ascendent serotonergic system projects in the cortex region, basal ganglia and limbic structures (amygdala and hippocampus). **(http://fblt.cz/en/skripta/regulacni-mechanismy-2-nervova-regulace/5-neurotransmisni-systemy/)**

Politis and Niccolini, 2015 described that PD results in the shortage of serotonergic terminals and are associated with the motor and non-motor complications.

Randhir *et al*., 2004 demonstrated that phenolic compounds are secondary metabolites in plants which are synthesized primarily through pathways of pentose phosphate, shikimate and phenylpropanoid. They are not frequent in algae, bacteria and fungi. Phenolic compounds are natural antioxidants which play a dominant role in human health and treatment of many diseases. Due to their antioxidant capacity, it provides protection against oxidative stress associated diseases. Sesamol (SES) (5-hydroxyl-1,3-benzodioxole or 3,4-methylenedioxyphenol) is a phenolic compound derived from the plant species *Sesamum indicum* L, which is a flowering plant belongs to the genus *Sesamum*. SES provides protection against multi-organ injury in animal model **(Hsu *et al*., 2006). Rangarajan *et al*., 2011** reported that SES has the capacity to restore the antioxidants in brain of glioma developed rats and showed anti-cancer property. **Hsu *et al*., 2007** demonstrated that SES inhibited the hydroxyl radical generation (Haber-Weiss reaction and Fenton reaction) in mice intoxicated with iron. These multidimensional health benefits of SES have driven us to study its potential on brain of rotenone-induced Parkinsonian models.

Materials and methods

Rotenone, L-DOPA, EDTA, Griess reagent and sesamol were purchased from Sigma-Aldrich (St.Louis, Missouri), USA. All other acids, bases, salts and solvents used were of highest purity and analytical grade.

Experimental animals:

Male Wistar albino rats (150-180 g) were used in the study. Animals were maintained at a temperature of 24±2ºC, in a 12 h dark/12 h light cycle, with food and water *ad libitum*. The studies were carried out with the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee of Sathyabama University, Chennai approved the protocol of the study (SU/CLATR/IAEC/VI/034/2016).

Experimental protocol:

The animals were divided into 5 groups, each containing 6 animals.

Group I: Vehicle (DMSO in corn oil intraperitoneal + Saline intraperitoneal) for 60 days.

Group II: Rotenone (3 mg/kg.B.wt intraperitoneal) for 60 days.

Group III: Co-treatment Rotenone (3 mg/kg.B.wt intraperitoneal) + Sesamol (50 mg/kg.B.wt intraperitoneal) for 60 days.

Group IV: Co-treatment Rotenone (3 mg/kg.B.wt intraperitoneal) + Sesamol (50 mg/kg.B.wt intraperitoneal) + L-DOPA (10 mg/kg.B.wt oral) for 60 days.

Group V: Co-treatment Rotenone (3 mg/kg.B.wt intraperitoneal) + L-DOPA (10 mg/kg.B.wt oral) for 60 days.

Preparation of test sample

The brains of experimental animals were homogenized with PBS (pH 7.4) and centrifugation was carried out at 2000 rpm and then the aliquots were used as test samples.

Analysis of neurotransmitters

Processing of tissue sample

The cold butanol was used for the homogenization of the tissue samples and a final concentration of 50 mg/ml was used. Then, centrifugation was carried out at 4^0C for 15 minutes. To the supernatant, 2.5 ml of n-heptane and 2.5 ml of distilled water were added. After mixing the contents thoroughly, the centrifugation was carried out at 1000xg for 5 minutes. To the aqueous phase, 200 mg of alumina, 1.5 ml of 2M sodium acetate was added and the pH was adjusted to 8.0 by 1N sodium hydroxide. The centrifugation was again carried out with the samples at 1000xg for 5 minutes. Later, 1.5 ml of supernatant was employed to analyze the neurotransmitters such as dopamine, epinephrine, norepinephrine and serotonin.

Estimation of dopamine, epinephrine and norepinephrine

The dopamine, epinephrine and norepinephrine in striatum of experimental animals were estimated by the protocol of **Kari *et al*., 1978.**

Statistical analysis

The statistical analysis was performed by SPSS version 20 from IBM. The results were expressed as mean±SD. One-way analysis of variance was applied to the data and the significance of the results was derived by running post hoc test. The $p<0.05$ were considered statistically significant. The graphs were plotted using Graph Pad Prism version 5.03.

Results and Discussion

Grace *et al*., 2007 revealed that dopaminergic neurons present in mid brain were responsible for the fundamental and complex structure of the brain to function. **Uchida *et al*., 2012** also observed that dopaminergic neurons in the mid brain are responsible for motor and autonomic functions. The long-term administration of L-DOPA induced several side effects due to elevated toxicity and inflammatory response **(Jolanta *et al*., 2014).**

ROT-induced animals (Group II) showed significant ($p<0.001$) decrease in the levels of dopamine, epinephrine, norepinephrine and serotonin in striatum **(figures 4-7)** when compared to vehicle-treated animals (Group I). ROT-induced animals treated with SES (Group III) ($p<0.01$), SES + L-DOPA (Group IV) ($p<0.001$), L-DOPA (Group V) ($p<0.01$) were noted with significant increase in the levels of dopamine, epinephrine, norepinephrine and serotonin when compared to ROT-induced animals (Group II). **Roghani and Behzadi, 2001** reported that the dopamine level in the brain can be retained by the supply of antioxidants. Decreased content and activities of dopamine and GSH was found respectively in ROT-induced PD model **(Hanan *et al*., 2004).**

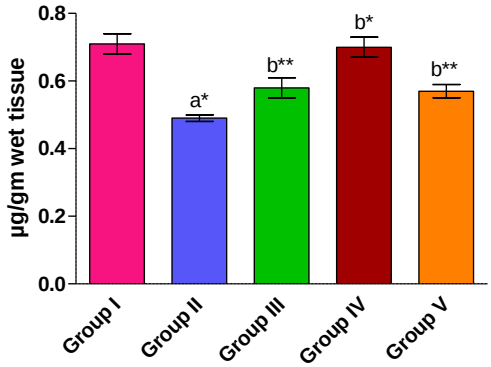

Fig 4: Level of dopamine in striatum of experimental animals

Values are expressed as mean±SD, n=6. Group I: Vehicle-treated animals, Group II: Rotenone-induced animals, Group III: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt), Group IV: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt), Group V: Rotenone (3mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt). Statistical significance: *p<0.001, **p<0.01, ***p<0.05, NS- Non Significant. Comparison: a – as compared with Group I; b – as compared with Group II.

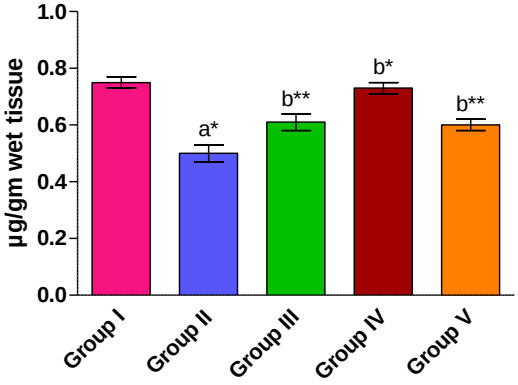

Fig 5: Epinephrine level in striatum of experimental animals

Values are expressed as mean±SD, n=6. Group I: Vehicle-treated animals, Group II: Rotenone-induced animals, Group III: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt), Group IV: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt), Group V: Rotenone (3mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt). Statistical significance: *p<0.001, **p<0.01, ***p<0.05, NS- Non Significant. Comparison: a – as compared with Group I; b – as compared with Group II.

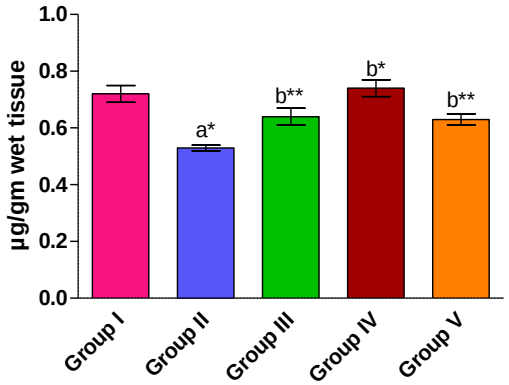

Fig 6: Level of norepinephrine in striatum of experimental animals

Values are expressed as mean±SD, n=6. Group I: Vehicle-treated animals, Group II: Rotenone-induced animals, Group III: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt), Group IV: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt), Group V: Rotenone (3mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt). Statistical significance: *p<0.001, **p<0.01, ***p<0.05, NS- Non Significant. Comparison: a – as compared with Group I; b – as compared with Group II.

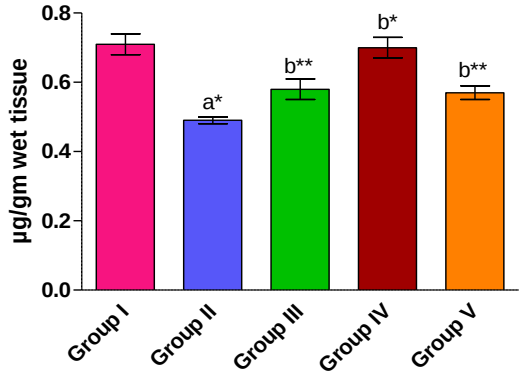

Fig 7: Serotonin level in striatum of experimental animals

Values are expressed as mean±SD, n=6. Group I: Vehicle-treated animals, Group II: Rotenone-induced animals, Group III: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt), Group IV: Rotenone (3mg/kg.B.wt) + Sesamol (50 mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt), Group V: Rotenone (3mg/kg.B.wt) + L-DOPA (10mg/kg.B.wt). Statistical significance: *p<0.001, **p<0.01, ***p<0.05, NS- Non Significant. Comparison: a – as compared with Group I; b – as compared with Group II.

Sawada *et al*., 2013 validated that catecholamines are potent in neurodegenerative diseases. Degeneration of dopamine and norepinephrine cause α-synuclein aggregation and plays a dominant role in the pathogenesis of PD. Degeneration of serotonergic axons is the general pathway in PD. The impairment in the serotonergic system and its projections forms α-synuclein with lewy neurites and lewy bodies. This protein dysregulation results in cytoskeleton variability, symptoms such as depression and anxiety **(Grosch *et al*., 2016). Wilson *et al*., 2018** noted that serotonergic system dysfunction also cause sleep disorders. In PD, sleep disorders comes under non-motor symptoms. Disturbance in sleep and arousal were observed with these defects.

Conclusion

The results conclude that alterations in neurochemicals plays the dynamic role in the development of PD. SES administration with ROT-induced PD has effective role in the prevention of abnormal modifications.

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Conflicts of Interests

Declared none

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