Evaluating neuroprotective effects in a *Drosophila* model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disease affecting around 0.8% of the global population with no cure to date. PD is characterised by the accumulation of Lewy bodies (LBs), which are caused by the aggregation of incorrectly folded α-synuclein (SNCA). Another form of PD manifestation is characterised by the loss of function of parkin, which encodes an E3-ubiquitin ligase. Despite extensive research, the cause of the onset and progression of PD remains unknown, and current therapeutics mainly help manage the disease. An alternative line of treatment can be useful. In the present study, we have employed two different genetic models of *Drosophila* UAS-SNCA and UAS-Parkin^{RNAi} to validate the neuroprotective effects of a mercury-based organo-metallic ayurvedic drug Ras-sindoor (RS). Our data indicate that the characteristic locomotory dysfunction phenotype of PD is alleviated upon administration of the RS. These flies also exhibit reduced transcript levels of initiator caspase, Death Regulator Nedd2-like caspase, resulting in reduced cell death of neurons. Additionally, RS-fed flies exhibit enhanced survival. The present studies thus highlight the importance of RS as a possible treatment drug for neurodegeneration mediated by PD.

Keywords: *Drosophila melanogaster*, Parkin, Parkinson's disease, Ras-sindoor, α-synuclein.

PARKINSON'S disease (PD) is the second most common age-related neurodegenerative disease after Alzheimer's disease, affecting at a frequency of over 150 people per 1,00,000 people worldwide, the number of people affected reaching approx 7,471,821 worldwide with about 3,88,194 deaths as of 2021 (ref. 1,2). The two major neuropathological features that characterise PD are: progressive loss of dopaminergic (DA) neurons in the *substantia nigra pars compacta* region of the midbrain, which affects the dopamine level in the striatum, and the presence of intra-neuronal Lewy bodies (LB), which are mainly the aggregation of α-synuclein (SNCA) protein. With the disease progression, PD patients develop four major motor symptoms: tremors, bradykinesia, muscle rigidity, and postural instability[3–5]. Most PD cases are sporadic, with an unknown etiology; it is possible that environmental or genetic factors are involved in the development of the disease. About 5–10% of cases of PD are familial and single gene mutations[6]. SNCA and parkin are two of the genes with mutations that have been linked to familial forms of PD. Wild-type and mutant forms of SNCA (A53T and A30P), which are presynaptic membrane proteins reportedly involved in the formation and release of synaptic vesicles, have been found to cause the early-onset autosomal dominant PD[7,8]. Mutations in parkin, which encodes an E3 ubiquitin ligase, have been found to be associated with autosomal recessive PD[9]. Apoptosis, mitochondrial dysfunction, oxidative stress due to SNCA accumulation, and parkin mutation have been reported to be major causes for loss of DA neurons[10–12].

To date, there is no effective cure or therapy to stop the progression of PD. Although there are several drugs being used for symptom management, such as levodopa, bromocriptine, pramipexole, ropinirole, rasagiline, entacapone, and selegiline, the majority of these drugs are enzyme inhibitors and hence are known to cause several side effects[13–15]. Therefore, the identification of new holistic therapeutic strategies is required that can target PD symptoms and slow down the disease progression.

Ayurveda, 'The Science of Life', is the world's oldest traditional system of medicine that originated in India around 5000 BCE[16]. The ayurvedic medicinal system also claims to promote healthy aging and is reported to alleviate symptoms of neurodegenerative disorders[17–19]. The classical manuscripts describing rasayanas and bhasmas form a branch of pharmacology known as rasashastra. Rasashastra broadly can be categorised into two medicinal forms, viz. Bhasmas of minerals and their combinations with herbs and raskalpa, also known as rasayanas. Further, they are classified as paarad/mercury-based and non-mercury-based preparations, depending on the inclusion of paarad/mercury and its sulphide, gandhak. A few examples of rasayanas are ras-sindur, siddha makardhwaja, bruhadvaat chintamani ras[20]. Bhasmas like Rajat Bhasma, Suvarna Bhasma, Sahasraputi Abhrak Bhasma, and Tamra Bhasma are prepared by combining different herbs for their purification, followed by the formation of bhasma after heating at a defined temperature in a furnace. The process

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