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Research Article

Evaluation of Neuroprotective effect of Bioactive compounds of *Mucuna monosperma* in PD lines of *Drosophila melanogaster*

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ABSTRACT

Parkinson's Disease (PD) is a progressive neurodegenerative disorder that affects the nervous system. Oxidative stress (OS) plays an important role in the degeneration of dopaminergic neurons in PD. Ongoing research is focused on discovering new plant sources and developing alternative methods of production to meet the increasing demand for the treatment of Parkinson's disease. Ethnopharmacognosy plays a vital role in bridging traditional knowledge with modern science, and screening natural compounds from edible plants is a valuable approach to discovering new therapeutic agents. Hence, the exploration of plants and their bioactive compounds for drug development is an ongoing and promising field. This paper embodies results on the neuroprotective property of *Mucuna monosperma* (MM). Two major bioactive compounds, namely l-3,4-dihydroxyphenylalanine (L-DOPA) and Ursolic acid, were identified and isolated by Prep-HPLC and Column chromatography. Further, the isolated compounds by HPLC. The antioxidant properties of crude, L-Dopa and Ursolic acid were scrutinized and verified through DPPH and Nitric oxide scavenging assay. To evaluate the neuroprotective property and anti-aging effect of bioactive compounds, negative geotaxis, courtship behavior, and longevity studies were conducted in the transgenic PD line of *Drosophila melanogaster* against oxidative stress status. The result reveals that the climbing ability, courtship behaviors and longevity were significantly increased in PD lines (Elva/SNCA-A30P) fed with bioactive compounds under stress conditions. Further it was noticed that synergetic effects of Ursolic acid along with L-Dopa showed excellent result.

Keywords: Parkinson's disease, Oxidative stress, *Mucuna monosperma, Drosophila melanogaster,* Negative geotaxis, Courtship behavior, longevity.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects the nervous system. It is characterized by a range of symptoms, including muscular rigidity, difficulty with balance and walking, depression and dementia. The exact cause of PD is not fully understood, but it involves the loss of dopamine-producing neurons in the brain.[1] Oxidative stress (OS) and neuro-inflammation have been identified as key factors for dopaminergic neuron death in case of PD.[2] OS is a physiological condition that occurs when there is an imbalance between the productions as well as an accumulation of reactive oxygen species (ROS) in tissues. It plays an important role in the degeneration of dopaminergic neurons in PD. Auto-oxidation of dopamine and neuromelanin production results in failure of dopaminergic neuron in PD patients that generates ROS and OS.

Plants have been used in traditional herbal treatments for centuries, and many of these plants contain a wide variety of secondary metabolites. The exploration of plants and their bioactive compounds for drug development is an ongoing and promising field. Ethnopharmacognosy plays a vital role in bridging traditional knowledge with modern science, and the screening of natural compounds from edible plants worldwide is a valuable approach to discovering new therapeutic agents. Antioxidant and anti-inflammatory agents have been shown to play a vital role in the survival of neurons and the alleviation of PD symptoms.[3] Various studies have shown the strong neuro-protective effect of different medicinal plant extracts for reducing PD signs due to antioxidant and anti-inflammatory properties.[4-6] Various phytochemicals such as thymoquinone, crocin, curcumin and polyphenols have been shown to have remarkable protective effects on the nervous system against oxidative stress and inflammatory responses.[7] One such medicinal plant having high scavenging activity is *Mucuna monosperma* (MM) belongs to the family *Fabaceae,* commonly known as negro beans or deer-eye beans, donkey-eye beans. It is a large woody climber with trifoliate leaves and possesses purple flowers. The plant MM has edible seeds enclosed in a restorative fruit pod that has unique medicinal properties due to its L-Dopa contents.[8] The seeds of clinical trials are a critical step in the process of verifying the effectiveness and safety of bioactive compounds. Many of the studies have not been carried out on the neuroprotective properties of MM through *in-vivo* analysis. In view of this present study has chosen to evaluate the neuroprotective property of bioactive compounds of MM by *in-vivo* studies using transgenic PD lines of *Drosophila melanogaster*.

MATERIALS AND METHODS

Chemicals

TLC plates were obtained from Merck, Germany. Silica gel, paraquat dichloride (PQ), ethanol,2,2-diphenyl-1-picrylhydrazyl (DPPH), chloroform, ethyl acetate, methanol, and were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), and Tris (Hydroxymethyl) Aminomethane-Hydrochloric acid (Tris HCl) were procured from Quali gens Fine Chemicals, Mumbai, India.

Preparation of Seeds Extract

The seeds of MM were collected from Koppa (Western Ghats), Chikkamagaluru District, Karnataka, India. The seeds were washed with water to remove the unwanted content present on them and dried under sunlight and in a hot air oven to remove moisture content. Further, the seeds were made in to a fine powder using an electric blender. The powder obtained was subjected to the soxhlet apparatus for extraction in methanol solvent.

Isolation and Characterization of Bioactive compounds.

Isolation of bioactive compounds from the crude extract of MM was made through preparative HPLC, column chromatography and HPLC.

Isolation of L-Dopa through preparative HPLC.

L-DOPA was isolated from the extract of MM as per the standard procedure. The mobile phase was made up of 0.1% formic acid in water as an aqueous phase (A) and methanol as an organic modifier (B), and it is delivered at a flow rate of 6 mL/min in the following gradient: initially % B is kept at 0.0% for 20 minutes; 20 to 30 minutes B is 10%; 30 to 40 minutes B% is 95; 30 to 31 minutes B% is 100 and 31 to 40 minutes 0.0B%. The sample was injected in a volume of 1000 µL. The column oven temperature was kept at an optimal level throughout the chromatographic run (22°C).

- **Mobile Phase-A:** 0.1% formic acid in water
- **Mobile Phase-B:** Methanol
- **Make:** Waters, USA
- **Model:** 2545 Binary gradient module
- **Injection volume:** 1000 µL
- **Column:** 250x10 mm, 5 µ particle size

Purification of L-DOPA by HPLC

The isolated compound L-DOPA from MM extract was then subjected to high-performance liquid chromatography (HPLC) analysis for identification and purity assessment. HPLC analysis was performed using the method described by Surwase et al. (2011).[9] It was carried out (Waters model no. 2690) on C8 column (symmetry, 4.6 mm X 250 mm) using methanol as a mobile phase with a flow rate of 1-mg ml^{-1} for 10 minutes and UV detector at 280 nm. The standard L-DOPA and Isolated L-DOPA reaction supernatant were prepared and injected into the HPLC column in HPLC-grade water. It was performed using the HPLC machine Shimadzu LC- Prominence 20AT with the column C18 column 250 x 4.6 mm, 5 µ particle

Identification and Isolation of Ursolic acid

- *Thin layar chromatography*

The methanol extract of the MM sample was subjected to a thin-layer chromatography (TLC) study as per the standard protocol.[10] The available TLC plates were coated with silica gel 60F 254 on aluminum sheets. Mobile phase 1 was Toluene: Ethyl acetate: Acetic acid (30:3:1) and Standard ursolic acid dissolved in dimethyl sulphoxide. The sample was dissolved in dimethyl sulphoxide and filtered before spotting. Chamber Saturation Time: 30 minutes.

- *Column chromatography*

Isolation of ursolic acid from MM's methanol extract was done using silica gel column chromatography. A 60 × 25 mm glass column was packed with silica gel (40 g, particle size 63–200 µm). Further, the crude extract was loaded onto the column and then eluted with different solvents, from ethyl acetate to hexane (from polar to non-polar solvent). TLC collected and analyzed ten fractions with a volume of 50 mL. Fractions containing Ursolic acid (Chloroform fraction) were pooled and concentrated under reduced pressure. Then, methanol crystallized the residue several times, and a white amorphous solid was obtained. Chloroform and methanol are the most effective solvents for uric acid isolation.

Purification of ursolic acid by HPLC

The isolated ursolic acid from MM extract was then subjected to the HPLC analysis for identification and purity assessment. The standard compound ursolic acid and the isolated compound ursolic acid from MM were analyzed by HPLC using the standard method.[11]

- Mobile Phase: Linear
- A: HPLC grade ACN (80%)
- B: HPLC water (20%)
- Flow Rate: 1-mL/min
- Injection volume: 10 µL
- Absorbance: 214 nm

Antioxidant Assay

MM's isolated L-DOPA and ursolic acid were screened for *in-vitro* antioxidant activity by Nitric oxide radical scavenging and DPPH radical scavenging activity.

Nitric oxide scavenging assay

Nitric oxide scavenging assay was performed as per the standard procedure.[12] Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Different concentrations of the MM extract samples were mixed with10 mM sodium nitroprusside in a phosphate buffer and incubated at 25°C for 180 minutes, then the mixture was added with freshly prepared Griess reagent that contained equal amounts of 1% sulphanil amide in 2.5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid. The sample without MM extract was considered as a control. The absorbance was measured at 546 nm using a microplate reader. The percentage inhibition of the extract was calculated and recorded. The percentage of nitrite radical scavenging activity was calculated using the following formula.

$$\text{Nitric oxide scavenged (\%)} = \frac{A \text{ control} - A \text{ test} \times 100}{A \text{ control}}$$

Where A control = absorbance of the control sample and Atest = absorbance in the presence of the samples of extracts or standards.

DPPH assay

The total antioxidant activity was measured using the DPPH radical scavenging method followed by Chan *et al.* (2007)[13] with slight modification. 1mg of MM extract was used for the estimation and mixed with 2 ml of DPPH solution. The assay was carried out on a 96-well plate. Methanol was used as a control and quercetin was used as standard (1-mg/mL). The mixture was shaken continuously and plates were incubated at 37°C for 30 minutes, kept in the dark for 30 minutes, then the absorbance of each solution was measured at 490 nm. The absorbance of the resulting solution was measured at 517 nm. The scavenging activity of each extract on DPPH radical was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{(1 - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

In-vivo Analysis

Fly culture melanogaster

To carry out *in-vivo* studies, genetic stocks of *D. melanogaster* has been used, P{UAS–Hsap\SNCA}, P{UAS-Hsap\SNCA.A30P}4, and Scer\Gal4$^{elav\text{-}C155}$ stocks were obtained from Drosophila Stock Center, Department of Zoology, Manasagangothri, University of Mysore, Mysuru, Karnataka. The flies were cultured in a standard wheat cream agar media seeded with yeast granules and maintained at 22 ± 1°C diurnal cycle (12 hours light and 12 hours dark) of relative humidity 70 to 80% and flies were maintained in 30 mL culture bottles. For all the analysis, synchronized 1st generation flies of *D. melanogaster* were used. To conduct further *in-vivo* analysis, flies were supplemented with the MM extract and bioactive compounds with 4 mg/mL.

Establishment of PD line of D. melanogaster

The PD line of *D. melanogaster* was developed using cross-experiments. The Virgin females of Elav-Gal4 strains were mated with the males of UAS-SNCA^{wt} and UAS-SNCA.^{A30P} The progeny expressed the human alpha-synuclein in the neurons and the flies are referred to as Elav/SNCA^{wt} as control and Elav/SNCA^{A30P} as PD flies. Negative geotaxis assay, courtship assay and longevity were carried out in F1 generation of Control and PD flies.

Induction of oxidative stress (OS test)

To know the neuroprotective potentiality in L-DOPA and ursolic acid of MM, flies were subjected to oxidative stress conditions. Paraquat dichloride (PQ) has been employed as OS molecule by following the method of Hosamane and Muralidhara.[14] About 15 days PD line flies were used for OS induction. The flies were starved in empty vials of size 9 x 3 cm for 2 hours. Then, flies were exposed to 15 mM PQ in 5% sucrose solution through a soaked filter paper for 2 hours duration. These flies were used for negative geotaxis, courtship experiments and longevity assay,

Negative geotaxis assay

The adult's locomotory function was assessed using the negative geotaxis assay. It was carried out as per the standard procedure.[15] The experiment was analyzed in 280 transgenic PD line flies, L-DOPA and ursolic acid-supplemented flies and OS-induced flies as well as PD lines with OS-induced ones. About 20 days, 20 adult males were released into a graduated empty plastic jar measuring 25 cm in length and 2 cm in diameter. The flies were given ten minutes to rest before being gently tapped to the bottom of the jar and allowed to ascend to the top. The number of flies per minute that climbed from the bottom to the 20-cm mark was tallied. The data was expressed as an average of four trials per replicate after the assay was run four times. The scoring procedure for the controls was the same.

- *Courtship assay*

The courtship assay was assessed as per the standard procedure.[16] Flies used for the courtship assay were kept in an opaque box. Taking into account the periodicity of *Drosophila* courtship behavior, the courtship assay was conducted in 20-day-old flies. Elav/SNCA^{wt} and Elav/SNCA^{A30P} unmated male was crossed with Elav/SNCA^{wt} and Elav/SNCA^{A30P} virgin female in a cylindrical transparent chamber (r = 1.5 cm, h = 0.5 cm) for 10 minutes or until copulation occurred. Recorded the courtship of each batch and 140 replicates were setup in the following batches. The control males and females, PD males and females with MM crude, L-dopa and L-dopa with ursolic acid, and PQ. The following essential features of male courtship were measured: (i) orientation, (ii) wing vibration, (iii) licking, and (iv) attempted copulation recorded the occurrence of copulation. In addition, recorded a novel behavioral parameter, nonsexual encounters (NSEs), encounters between the male and female flies that did not lead to sexual activity.

- *Longevity*

A longevity experiment was carried out as per the procedure of Yoon *et al.,* (1990).[17] The studies have been carried out in MM extract fed-batch and bioactive compounds of MM-supplemented flies of PD line of *D. melanogaster*. It was conducted in virgin flies. Newly emerged flies were collected within 24 hours and 20 flies were released to each experimental culture vial of size 9 x 3 cm, containing MM crude extract in one group L-DOPA with Ursolic acid in another group and wheat cream agar media. Yeast-seeded groups were considered as control. For every 3 days, flies were transferred to fresh culture vials and exposed to paraquat on the 15th day for 2 hours. The vials were checked daily for observation of mortality in flies and the same was recorded for each fly. Regular monitoring of the vials is sufficient to determine the viability of the flies. About 280 such replicates were maintained, and flies were maintained at 22 ± 1°C.

Statistical Analysis

All data are presented as mean ± standard errors of three separate biological samples. The data (mean±SE) was analyzed by one-way and two-way ANOVA followed by 'DMRT' a post-hoc test using SPSS version 20.0. The *p-value* of 0.05 was considered the minimum significance level ($p < 0.05$).

RESULT AND DISCUSSION

Neurodegenerative diseases are frequently associated with a progressive loss of movement ability, reduced life span, and age-dependent neurodegeneration. *Drosophila* PD models can be helpful to explore the therapeutic management of the disease against drugs. The neuroprotective potentials of certain medicinal natural herbs (*Bacopa monnieri, Mucuna pruriens, Withania somnifera, Curcuma longa, Gingko biloba, and Camellia sinensis*) has been reported[18] and proved their novel therapeutic strategies against PD. L-dopa is a precursor of dopamine used as the most effective symptomatic drug treatment for Parkinson's disease. Most of the isolated L-dopa is synthesized chemically. The available synthetic L-Dopa has many negative secondary effects on the systems. Various natural sources produce L-Dopa from different plants, and they have advantages compared to chemical methods due to its low-cost approach. It reduces the secondary effects and helps slow down the disease's progression. Plants belonging to the Fabaceae family contain significant amounts of L-Dopa. *Mucuna* seeds, a rich source of naturally occurring L-dopa, contain about 4 to 7%. It has been reported that *Mucuna pruriens* seeds are a rich source of L-Dopa[19] and have been used traditionally as an effective remedy for several brain-related problems, including Parkinson's disease. Due to the limitations of human genetic analysis, all the studies have been performed in model organisms, including mice, fruit flies, and worms as well as in cell culture. Hence, the fruit fly *Drosophila* has emerged as a valuable model for studying mechanisms of human neurodegenerative diseases, including PD. *Drosophila* adult negative geotaxis behavior, as an indication for possible motor defects associated with neurodegeneration.

Methanolic extract of Mucuna containing low levels of L-dopa showed anti-PD effects including improvements of motor function and olfactory response in a *D. melanogaster* genetic model of PD[20]. Further, they reported that the anti-PD effects of the *Mucuna* was due to the result of other compounds beyond L-dopa alone. Recent studies also suggest that phytochemicals apart from L-dopa may also contribute to the overall neuroprotective activities of *M. pruriens*.[20,21] The alternative plant for the rich source of L-Dopa is *M. monosperma*. Therefore, in this study, L-Dopa and Ursolic acid from the methanolic seed extract of *M. monosperama* (MM). L-Dopa was characterized, isolated, and purified from the preparation HPLC method using standard L-Dopa (Figs. 1 and 2). It is a dihydroxyphenylalanine (DOPA), a flavonoid compound having a large neutral amino acid that is the precursor for dopamine. Similarly, another flavonoid compound, ursolic acid, was characterized by TLC, isolated and purified from column chromatography (Fig. 3). Flavonoids are preventive or therapeutic agents against the loss of DA neurons, which are vulnerable to oxidative stress, in different models of PD.[22] The potent antioxidant properties of L-Dopa and ursolic acid of MM has been evaluated through DPPH and Nitric oxide radical inhibition assay, and further evaluation of their neuroprotective effects has been carried out in vivo bioassays in PD model of *D. melanogaster*.

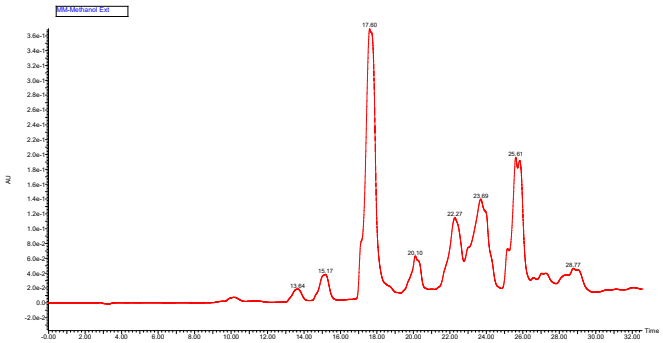

Fig. 1: Prep-HPLC result of separating L-dopa from methanolic extract of MM seed

Antioxidant Activity

The result of antioxidant activity by DPPH method and Nitric oxide scavenging activity is shown in Table 1. The activities were measured in total extract, isolated bioactive compounds viz. L-dopa and ursolic acids. The DPPH activity was found to be high in a crude extract of MM (130.34 ± 0.03 µg/mL). The activity in Ursolic acid was 89.11 ± 0.02 µg/mL, and the activity was least in L-dopa (35.25 ± 0.03 µg/mL). The DPPH activity increased by 60.44% in ursolic acid compared to L-Dopa. A similar observation was noticed in the nitric oxide radical inhibition study. In the crude total extract, it was 183.79 ± 0.05 µg/mL, least in L-Dopa (53.13 ± 0.03 µg/mL). The activity was increased by 19.18% more in ursolic acid than L-Dopa. Further, it was noticed that both antioxidant activities were more in a crude extract of MM due to the synergetic effects of various other bioactive compounds. Among the two bioactive compounds analyzed, urasolic acid was the potent antioxidant.

In-vivo study

Antioxidants are ideal therapeutic approach for the management of PD. *Drosophila* has already proven to be an excellent model in Parkinson's disease due to high degree of conservation in neuronal

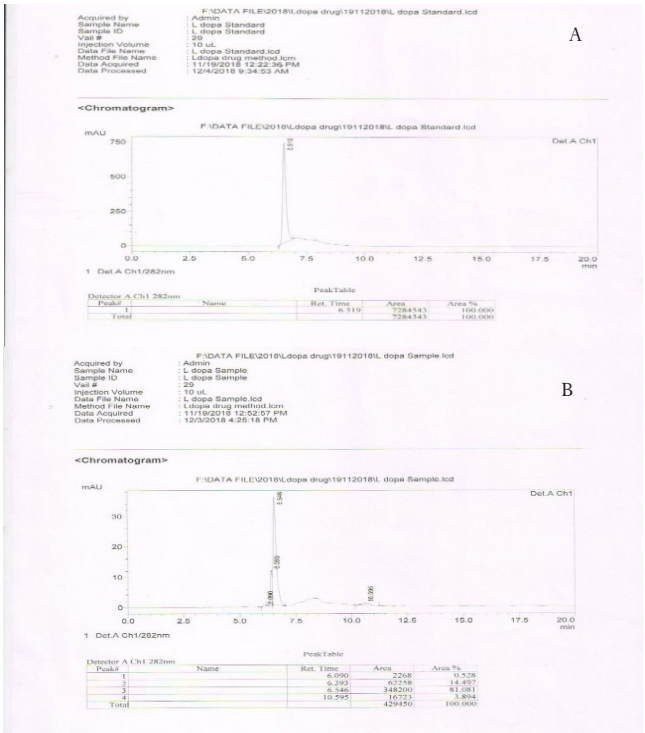

Fig. 2: The result of HPLC for (A) Standard L-DOPA and (B) L-DOPA extract of MM

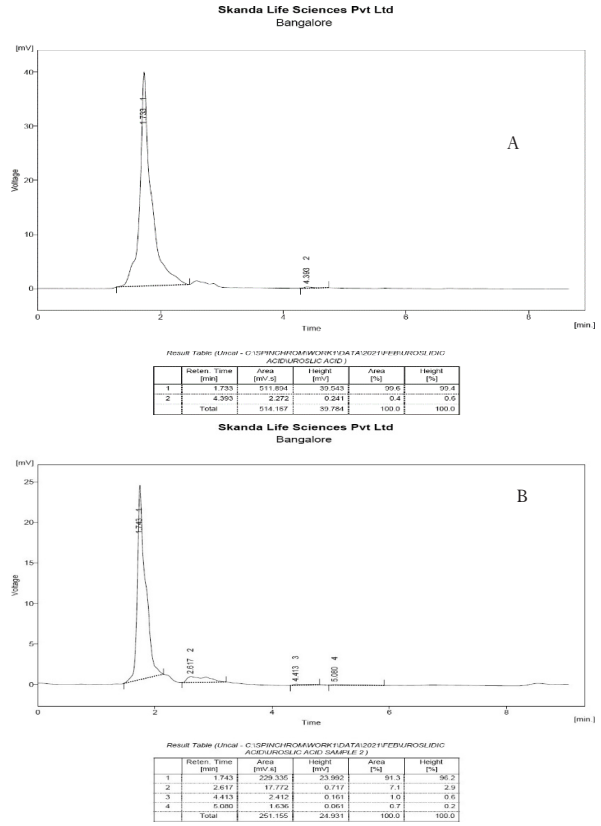

Fig. 3: Result of HPLC for (A) standard ursolic acid and (B) Ursolic acid extract of MM

function. The present study assessed negative geotaxis assay, courtship behaviors and longevity in PD lines and bioactive compounds of MM-supplemented flies under stress conditions.

Negative geotaxis

Negative geotaxis is an innate escape response, which is a natural tendency of flies to move against gravity for specific stimuli.[15] The climbing assay is useful in the study of neurodegenerative disorders such as Parkinson's disease. It is a simple, cost-effective assay that measures the motor ability of an organism. Locomotion is a tightly regulated neuronal function. The locomotion (climbing) assay is commonly used for assessing the behavior of flies expressing amyloidogenic proteins in their brain and also used to assess the neural dysfunction caused by the flies due to the expression of mutant UAS-A30P α-syn in the nervous system.[23]

Table 1: Antioxidant activity of MM crude, L-DOPA, and ursolic acid by DPPH method and nitric oxide scavenging assay

Test compound	*DPPH inhibition IC_{50} values Mean ± S.E (μg/ml)*	*Nitric oxide radical inhibition IC_{50} values Mean ± S.E (μg/ml)*
MM *Crude* extract	130.34 ± 0.03[a]	183.79 ± 0.05[a]
MM- L-DOPA	35.25 ± 0.03[b]	53.13 ± 0.03[b]
MM -Ursolic acid	89.11 ± 0.02[c]	65.74 ± 0.03[c]

Note: The groups with mean values having different letter in the parenthesis are significantly different at the 5 % level according to DMRT.

Negative geotaxis behavior is based on the flies being tapped to the bottom of the cylinder. Normal synchronized flies are successfully climbed over a 2 to 5 cm height in 10 to 20 seconds. The result of the negative geotaxis assay is represented in Fig. 4. The negative geotaxis assay was conducted in treated batches to compare the climbing ability of PD and control flies. In the present study, the assay revealed that more than 80% of the flies in the control group (Elav/SNCA) showed movement to the top of the graduated column. The climbing ability in the control fly was considered as 100%, and the climbing ability of other tested groups with the SNCA. The OS-induced Control (SNCA) flies showed a climbing ability of 23.33%. The climbing ability of PD line flies was low (36.67%), but the remarkable least climbing activity was observed in OS-induced PD line. In contrast, the climbing ability was increased in OS-induced PD line fed with crude extract of MM and bioactive compounds (L-Dopa and Ursolic acid). The highest climbing ability was noticed in OS-induced fed with a combination of L- Dopa and ursolic acid (66.67%). This significant increase of locomotory behavior is due to the combination effect of L-Dopa and Ursolic acid. This synergistic effect of components of MM showed a positive response in PD flies even under stress conditions.

Courtship assay

Drosophila males display complex behaviors that have evolved to achieve reproductive success.[24] Courtship in *Drosophila* is used to screen genes linked to Parkinson's Disease in humans. Hence, it is considered as more precise model for Parkinson's Disease.[16] The courtship ritual of the male fly exhibits a series of behaviors to get the orientation toward the female such as wing extension and copulation. Tapping a female fly with his forelegs, contacting her genitalia with his mouthparts, singing a species-specific courtship song, and bending his abdomen to copulate. The present study performed a courtship behavior assay in 20 days. The control PD line was treated with crude extract of MM and bioactive compounds (L-Dopa and Ursolic acid) against PQ. The result of the courtship assay was given in Fig. 5. Sexual activity was normalized to consider SNCA behavior as ideal (100% sexual activity). All seven courtship activities were decreased in PD line, OS-induced PD line and OS-induced control groups. The licking behavior was least in OS induced PD line (6.67%). However, when OS-induced PD flies, supplemented with MM extract, L-Dopa alone and a combination of L-Lopa and Ursolic acid showed a gradual increase of all the behavioral activities. Further, the data reveals that Courtship activity was increased by 4.75 times more in OS-induced PD line flies after being supplemented with L-dopa with Ursolic acid. PD males performed less sexual activity (up to 50% reduction related

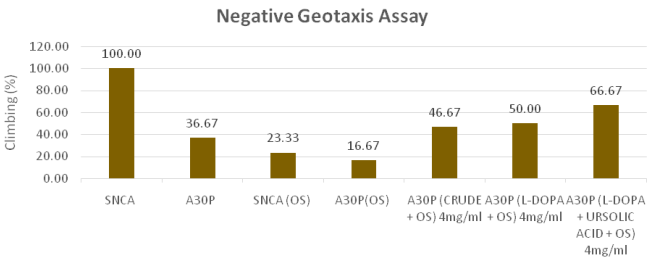

Fig. 4: Negative geotaxis behavior of transgenic line of PD *Drosophila* supplemented with MM crude, L-DOPA and L-DOPA with Ursolic acid against PQ

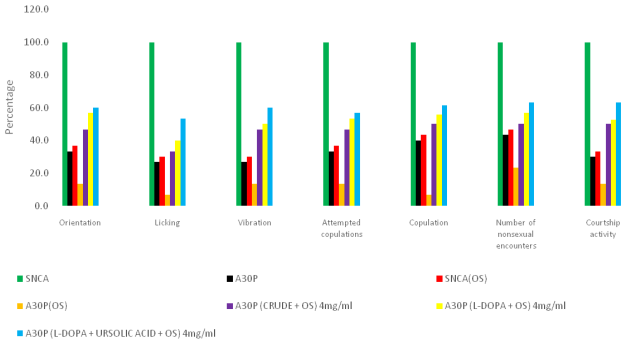

Fig. 5: Courtship behavior of 20 days control and PD flies are evaluated by 7 parameters of sexual activity treated with crude of MM, L-dopa and L-dopa with Ursolic acid extract against PQ

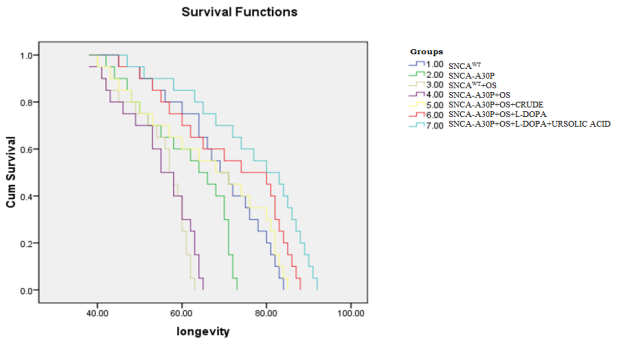

Fig. 6: Survival Curve of transgenic PD line of male *Drosophila* supplemented with crude MM, L-DOPA and L-DOPA with ursolic acid against PQ

to Control activity) in all sexual parameters. The overall result suggests that PD line males were impaired in sexual focus, ability to follow females, and coordination. All the behavior was improved after treatment with L-Dopa and ursolic acid.

Longevity

Longevity is the total duration of lifespan. The supplementation of various antioxidant-rich medicinal plant extracts increases the longevity in *D.melanogaster*. It has been proved that the supplementation of acai pulp, *Asparagus racemosus, Phyllanthus* species[25] extends the lifespan of *D. melanogaster*. The flavonoids and polyphenols found in cocoa significantly increased the mean lifespan of *D. melanogaster*.[26] Curcumin, a bioactive polyphenolic compound extracted from *Curcuma longa*, has been shown to significantly increase fruit flies' lifespan by 25.8%.[27] Life expectancy increased significantly with L-Dopa therapy. The administration of high concentrations of L-dopa to mice prolongs the mean lifespan by 50%.[28] The prolonged survival was evident when the patients were treated during the early stage of the illness.[29]

Paraquat exposure caused reduced lifespan in flies as well as movement disorders. In the present study, longevity experiments were carried out in different batches under stress conditions to know the effect of crude methanolic extract of MM, isolated bioactive compounds L-dopa and Ursolic acid on the life span of PD flies in *D. melanogaster*. The oxidative stress was induced by paraquat. Elav/SNCA line was considered positive control, and Elav-SNCAA30P, a PD line was considered negative control. The survival curve shows the longevity data (Fig. 6). The mean lifespan of the control was 79.4 ± 0.4 in males 80.2 ± 0.66 in female groups. PD line showed decreased longevity than its control. Mean lifespan was decreased by 26.29% in male groups while decreased by 33.92% in female groups. When OS was induced to control, and PD flies, longevity was reduced. In the male group, lifespan was reduced after OS induction by 46.60%, while in female groups, it was reduced by 53.12%. A similar observation was noticed in PD line with OS induction. The mean life span was reduced by 51.38% in the male group and by 54.86% in the female group. Further, it was noticed that when OS-induced PD flies were supplemented with MM extract, L-Dopa, L-Dopa, and ursolic acid, longevity was increased in both male and female grouped batches. The highest longevity increase was observed in OS-induced PD flies, supplemented with both L-Dopa and ursolic acid. Mean lifespan was increased by 53.36% in males 58.01% in females. The increased lifespan was due to the synergetic effect of both L-Dopa and Ursolic acid.

Park *et al.*, (2012)[30] have studied the efficacy of the Curcumin of *Curcumin longa* as a therapeutic agent in reducing PD symptoms. Further, they showed that curcumin improves mobility defects of *Drosophila* exposed to acute PQ toxicity. Siddique *et al.*,(2014)[31] have studied the effect of curcumin on lifespan, activity pattern, and oxidative stress in the PD model flies and reported that curcumin increases the life span of PD model flies significantly with delay in the loss of activity pattern, reduction in the oxidative stress. In the present analysis, L-Dopa and ursolic acid of MM increase lifespan and improve the climbing ability and courtship behaviors in PQ-induced PD flies.

CONCLUSION

Oxidative stress is a crucial step that causes the onset of PD. In the present study, OS-induced PD flies, after being supplemented with L-Dopa and Ursolic acid, exhibit improvisation in climbing ability and courtship behaviors. There is an association between PD and lifespan. Analyzing two bioactive compounds increases the lifespan of Oxidative stress-induced PD flies. Due to this potential of neuroprotective activity of MM, they may have a positive impact on aging by extending lifespan in PD flies even under stress status. The data from the *in-vitro* and in vivo studies proves the neuroprotective and anti-PD properties of these two active compounds of MM. The present study also suggests that the strong antioxidants L-Dopa with ursolic acid help reduce the oxidative stress generated in PD lines of *D. melanogaster*. However, the amount of the bioactive compound L-Dopa are more in *M. monosperma* than *in M. puriens* and *M. gigantean*,[32] can be used as a potential pharmacological candidate for treating neurodegeneration in PD and should be investigated further in pre-clinical studies.

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