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Original Article

Carotenoid pigment from *Micrococcus luteus* confers neuroprotection in a cellular model of Parkinson's disease via BDNF upregulation and oxidative stress reduction

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ABSTRACT

Background: Microorganisms produce a wide range of natural pigments with important bioactive properties. Carotenoid pigments from *Micrococcus luteus* (*M. luteus*) have been identified as potential neuroprotective agents. This study aimed to investigate the protective effects of *M. luteus* pigment in a cellular model of Parkinson's disease.

Methods: Carotenoid pigment was isolated from wild strains of *M. luteus* and structurally characterized. Its neuroprotective activity was assessed in SH-SY5Y neuroblastoma cells exposed to rotenone. Cell viability, reactive oxygen species (ROS) scavenging, and autophagy modulation were evaluated. Additionally, mRNA expression levels of brain-derived neurotrophic factor (BDNF), a key therapeutic target in Parkinson's disease, were quantified.

Results: *M. luteus* was identified using MALDI-TOF MS, yielding a score of 2.31. The purified yellow pigment exhibited FTIR peaks at 3269.07 cm^{-1}, 1629.79 cm^{-1}, and 1015.55 cm^{-1}, which align with the characteristics of hydroxylated carotenoids (xanthophyll type). In SH-SY5Y cells, rotenone decreased viability to less than 50%, while co-treatment with pigment restored viability in a concentration-dependent manner, reaching over 90% at 25 µg/mL. Rotenone reduced neurite length by approximately 75%, whereas it was maintained at a concentration of 25 µg/mL pigment. Flow cytometry demonstrated reduced LC3 intensity in the presence of rotenone, which was subsequently restored by co-treatment with pigment, suggesting an increase in autophagy. Rotenone resulted in a marked elevation of ROS levels, whereas pigment treatment significantly reduced these levels in the cells. Rotenone inhibited BDNF expression, whereas co-treatment with pigment restored and elevated BDNF levels to nearly control values.

Conclusion: The carotenoid pigment from *M. luteus* demonstrates significant neuroprotective activity by improving cell viability, scavenging ROS, and enhancing BDNF expression in a Parkinson's disease cell model. These findings highlight its potential as a natural therapeutic candidate for neurodegenerative disorders.

1. INTRODUCTION

Owing to their antimicrobial [1], antioxidant [2], and anti-tumor activity [3], pigments from soil-derived bacteria find application in the pharmaceutical industry [4]. Studies have shown that microbial

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pigments, such as carotenoids, canthaxanthin, astaxanthin, and melanins, including phycocyanin, possess anticancer, anti-inflammatory, and antioxidant properties [5, 6]. Xanthophylls, hydroxylated carotenoids, have more polarity than carotenes, hence increasing membrane interactions and antioxidant efficacy. Xanthophylls, in contrast to carotenes that mainly serve as vitamin A precursors, have a role in neuroprotection, membrane stability, and the control of stress-response pathways [7]. Due to the detrimental consequences of synthetic pigments, natural pigments are gaining relevance nowadays. Increasing demand for natural pigments is a result of their beneficial impacts on both human and environmental health [8].

Gram-positive, aerobic coccoid organisms of the genus *Micrococcus* are abundant in carotenoid pigments, recognized for their bioactive and radioprotective qualities. Although the carotenoid pigments generated by *Micrococcus* show beneficial effects in the food, pharmaceutical, cosmetic, and dye industries [9, 10], there is no scientific or systematic investigation into their neuroprotective effect.

According to the conclusions drawn by the World Health Organization (WHO), it is anticipated that by the year 2040, neurological disorders will become the second leading cause of mortality [11]. Neurodegenerative disorders are a heterogeneous group of incurable illnesses like Alzheimer's disease (AD) and Parkinson's disease (PD) that affect neurons, causing progressive deteriorating changes in the structure and function of nerve cells. The WHO recognizes AD as a global public health priority [12]. In the case of PD, prevalence and incidence rates are increasing rapidly [13]. Currently available treatments of neurodegenerative diseases are less efficient in curing and can only manage the symptoms or slow progression of the disease. Using natural products is a better therapeutic approach for these diseases, minimizing side effects [11]. Investigations within this domain will doubtlessly facilitate the advancement of prospective therapeutic strategies capable of modulating inflammation and substantially mitigating neuronal damage [14].

In light of this, we investigated the neuroprotective effect of a carotenoid pigment extracted from the soil microbe *Micrococcus luteus* (*M. luteus*) in SH-SY5Y neuroblastoma cell lines subjected to rotenone induced degeneration.

2. METHODS

2.1 Separation of pigment-producing soil microorganisms

Samples of soil were collected from different locations in Thiruvananthapuram district, Kerala, India. The types of soil consisted of fairly rich brown loam of laterite and sandy loamy soil. Collected about 25 g of soil, removed the debris from the surface, and preserved it in an icebox prior to transportation to the laboratory. Strains that produce pigment were isolated from the soil by the spread plate method. Colonies exhibiting pigmentation were carefully subcultured and grown in Micrococcus differential agar (using mannitol salt agar and following ASM guidelines), and yellow pigment producers were selected for further studies.

2.2 Bacterial identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis

Bacteria were detected by MALDI-TOF MS analysis. A fresh bacterial colony was directly smeared onto a stainless-steel MALDI target plate. The spot was overlaid with 1 μL of 70% formic acid to facilitate protein extraction. After drying, 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was added. The prepared target plate was inserted into the Bruker Microflex LT™ instrument (Bruker Daltonics GmbH & Co. KG, Germany). Spectra were acquired in positive linear mode over a mass range of 2,000–20,000 m/z. Acquired spectra were analyzed using Bruker's Biotyper software. Identification was based on comparison with the Bruker reference database.

2.3 Bacterial pigment extraction and purification

M. luteus was incubated in specific growth media (Tryptone -10 g, Yeast extract -1.0 g, Dextrose -10 g, Bromocresol purple -0.040 g in 1000 mL distilled water) at room temperature and incubated for 72 h for pigment production. Bacterial cells were then separated by centrifugation at 8000 rpm for 10-15 min, and the supernatant was collected for further pigment isolation. Then, the supernatant was extracted with chloroform-to-supernatant ratio of 1:2 (v/v) for 48 h in a separating funnel. The pigments were carefully separated, dried under pressure, and subjected to further studies.

The pigment generated by *M. luteus* was purified via column chromatography employing silica gel (60-120 mesh size), and initially eluted with n-hexane at a flow rate of 1 mL/min. The solvent's polarity was subsequently enhanced by the addition of ethyl acetate (5-100 %), and the yellow-hued fractions were collected from the column.

2.4 Characterisation by FTIR spectroscopy

The pigment extracted and purified from *M. luteus* was analyzed using FTIR spectroscopy, as detailed by Pawar and coworkers [15]. Potassium bromide was used to concentrate and pellet the pigment's methanolic extract. The comparative intensity of light that is transmitted by the pigment was assessed in relation to the absorption wavelength within the range of 400 to 4000 cm^{-1} using FTIR spectrophotometer (Nicolet™ iS50 FTIR Spectrometer, Catalogue number 912A0760, make Thermoscientific, USA).

2.5 Cell Lines

Neuroblastoma cells, SH-SY5Y (ATCC Cat# CRL-2266, RRID:CVCL_U924) were obtained from the National Centre for Cell Sciences (NCCS) in Pune, India, and cultured in Dulbecco's Modified Eagle's Medium (DMEM). Cells were maintained in a culture flask containing appropriate antibiotic solution, with DMEM, enriched with L-glutamine, 10% FBS, and sodium bicarbonate. Cell culture was sustained at a temperature of 37°C within a 5% CO_2 incubator. Confluent layer of cell culture was then trypsinized with 0.25% trypsin-EDTA, reseeded at 1 x 10^5 cells/mL of DMEM with 10% FBS after being washed with phosphate-buffered saline (PBS), and incubated overnight.

2.6 Neuroprotective property of the pigment

2.6.1 Cell morphology and Cell viability

Cells were pretreated with 10 µM rotenone [16] in a 96-well culture plate (5 x 10^3 cells/well) and were incubated at a temperature of 37ºC within a humidified incubator supplemented with 5% CO_2 for a period of one hour. Cells were treated with pigment extract having concentrations from 6.25 to 100 µg/mL dissolved in 0.1% dimethyl sulphoxide (DMSO) and incubated for 24 h. Morphology was then studied by using the MTT assay [17]. A 30 µL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was administered to cells exposed to varying concentrations of pigment, ranging from 6.25 to 100 µg/mL. Following a PBS wash, the cells were incubated at 37 °C for 3 hours, then washed with PBS, treated with 200 µL DMSO, and incubated at room temperature for 30 minutes until the cells were lysed and a homogeneous color was achieved. Following a 2-minute centrifugation, the absorbance was measured at 540 nm, utilizing DMSO as a blank to determine the viability percentage. The experiments were done in triplicates, and the results are represented as mean ± SD.

After examining the cell viability, cells were treated with 10 µM rotenone for one hour. After incubation, 25 µg/mL of the pigment was added and kept in an incubator for 24 h. Cells serving as controls were also kept, and further experiments were carried out.

2.6.2 Reactive Oxygen Species

Cells were seeded onto 96-well plates at a density of 5000–10,000 cells per well. Reactive oxygen species were analyzed using a standard protocol [18]. After treatment, cells were washed with PBS, treated with 100 µM dichloro dihydro fluorescein diacetate (DCFDA) diluted in DMEM and 1% FBS for one hour, rinsed the cells twice using PBS and examined underneath a fluorescent microscope at 470 nm excitation and 635 nm emission wavelength to measure the fluorescence. DCF fluorescence was imaged on a fluorescent microscope (Olympus CKX41, Japan), and relative intensity was measured using the ImageJ analysis software.

2.6.3 Neurite length

Cells were seeded in 24-well plates at a density of 5 × 10^4 cells/well and allowed to attach overnight and differentiated using 10 µM all-trans retinoic acid (RA) for 5 days, with media replacement every 48 hours. After differentiation, the cells were treated as per the method described previously, and following incubation, the cells were stained with 5 mg/mL fluorescein diacetate (FDA) in the dark for 15 minutes to visualize live neurites. Cells were then gently washed with PBS and observed under a fluorescence microscope using a blue filter (excitation ~490 nm, emission ~520 nm). Neurite outgrowth was assessed by measuring neurite lengths using imaging software, and results were expressed relative to untreated control cells [19].

2.6.4 Autophagy assay

Cells were seeded in 6-well plates at a density of 2 × 10^5 cells/well. The Cyto-ID® autophagy detection kit was used to detect autophagy. Cyto-ID® green detection reagent was diluted using 1 x assay buffer to prepare the dye stain solution. Each sample received a small amount of diluted green stain

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Table 1. The primer sequences used in qPCR

Oligo name	Forward		Reverse	
	Sequence (5' - 3')	**Tm**	**Sequence (5' - 3')**	**Tm**
GAPDH	ACTCAGAAGACTGTGGATGG	57.3°C	GTCATCATACTTGGCAGGTT	55.3°C
BDNF	AGCTGAGCGTGTGTGACAGT	59.4	ACCCAATGGGATTAACACTTGG	57.3

solution, thoroughly mixed, and kept in an incubator for a duration of 30 min at 37°C in the dark. Subsequently, the cells were subjected to centrifugation and then rinsed with buffer. Reconstituted the pellets in the buffer and performed flow cytometric analysis with Muse Cell Analyzer (Luminex Corporation, USA), and further evaluation was conducted via Muse analytic software.

2.6.5 Gene Expression Study of Brain-Derived Neurotrophic Factor (BDNF)

Real time polymerase chain reaction (qPCR) was performed to assess BDNF gene expression. Trizol was used for extracting total RNA as per the manufacturer's instructions, and Qubit 3. cDNA Synthesis Kit was used for cDNA synthesis in a thermal cycler. Twenty minutes of cycling at 42ºC and 5 minutes at 85ºC were performed to conduct qPCR analysis. All tests were carried out three times, and the results were evaluated using the ΔΔCt method. The primer sequences utilized [20, 21] are displayed in Table 1

2.7 Statistical analysis

Statistical analysis was performed using GraphPad Prism Software 5.01 (GraphPad Software, Inc., San Diego, CA). The data were represented as mean ± SD (n = 3) and analyzed by one-way analysis of variance (ANOVA), and P <0.05 was considered statistically significant.

3. RESULTS

3.1 Isolation of bacteria and bacterial pigment

M. luteus was effectively isolated from samples of soil obtained from various locations in Thiruvananthapuram (Figure 1). The recognition of the microorganisms was validated through MALDI-TOF MS analysis. The target plates were placed into the Bruker Microflex LTTM apparatus. Positive linear mode was used to acquire spectra from 2,000 to 20,000 m/z. We used Bruker's Biotyper software to analyze the acquired spectra. With a score of 2.31, it is highly probable that the specimen was identified at the species level as *M. luteus* through comparison with the Bruker reference database.

Figure 1: *Micrococcus luteus* grown on *Micrococcus* differential agar.

Yellow pigment was isolated and purified using column chromatography and characterized by FTIR (Figure 2). Peaks were obtained at 3269.07 cm^{-1}, 1629.79 cm^{-1}, and 1015.55 cm^{-1}. The presence of hydroxyl functional groups, as shown by the peak at 3269.07 cm^{-1}, suggests the potential presence of oxygenated carotenoids, such as xanthophylls. This peak provides more evidence that *M. luteus* contains carotenoids of the xanthophyll type. The characteristic feature of carotenoids, the presence of conjugated double bonds, is indicated by the peak at 1629.79 cm^{-1}. Showing the pigment-containing long polyene chains. Once again, the peak at 1015.55 cm^{-1} suggests that *M. luteus* contains hydroxylated carotenoids, as it reflects skeletal vibrations in the polyene chain and may also indicate C-O stretching. The FTIR results strongly suggest that the *M. luteus* pigment is a carotenoid, more especially an oxygenated type like xanthophylls.

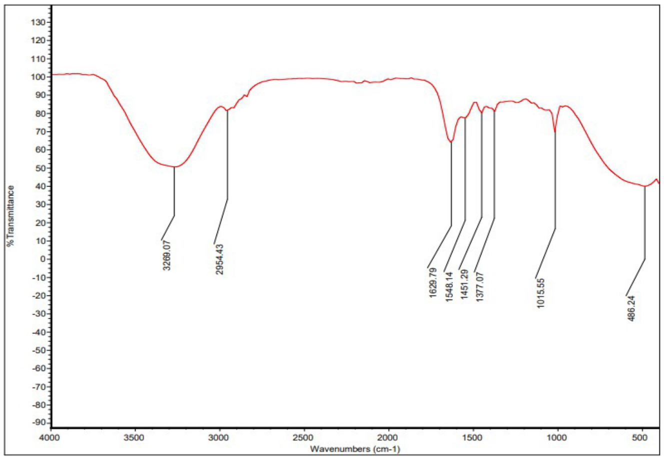

Figure 2: FTIR analysis of the spectrum for the pigment extracted from *M. luteus*

3.2 Neuroprotective activity of pigment induced by rotenone in SH-SY5Y cells - MTT Assay

Figures 3A and 3B demonstrated improved cell viability when cells were subjected to concurrent treatment with the pigment and rotenone. A comparison was made to those cells treated exclusively with rotenone. More than 90% cell viability was seen in cells exposed to 25 µg/mL of pigment along with rotenone. Cell viability was found to be concentration dependent and reached a maximum at 25 µg/mL and then started declining. Cells exposed to rotenone alone showed less than 50% cell viability. The results of the MTT assay confirmed the neuroprotective activity of the pigment. Morphological characteristics of cells subjected to 25 µg/mL pigment treatment, along with rotenone, were found to be comparable to those of the control. Based on these observations, we selected this concentration for further studies.

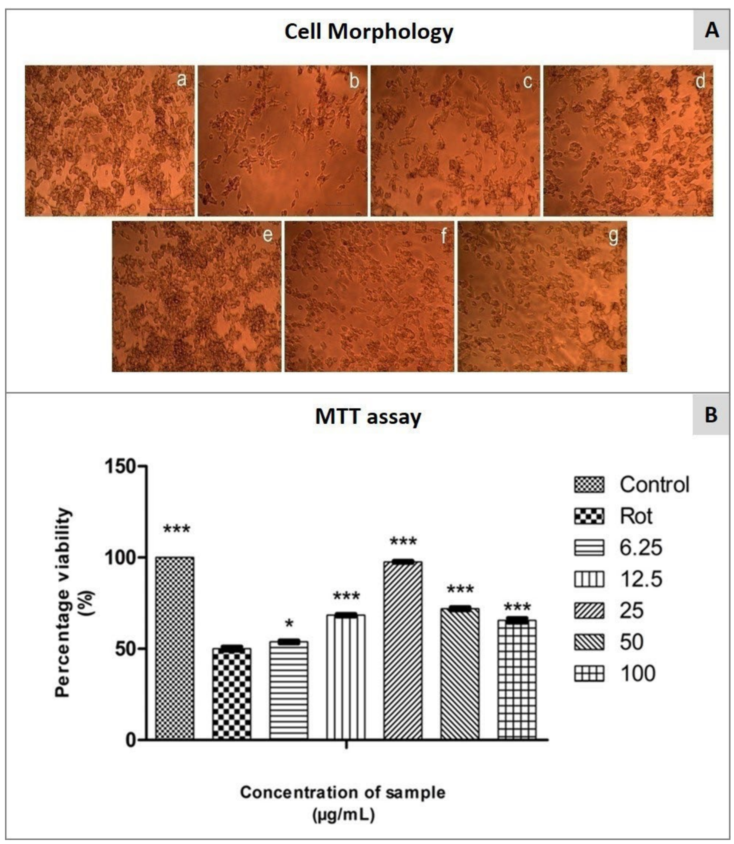

Figure 3: Neuroprotective activity of pigment against rotenone-induced toxicity in SH-SY5Y cells. (A) Photomicrographs (10X magnification) showing the *in vitro* neuroprotective effect of pigment on rotenone-induced SH-SY5Y cells: (a) untreated control, (b) rotenone-exposed cells, and rotenone-exposed cells co-treated with isolated pigment at concentrations of (c) 6.25 µg/mL, (d) 12.5 µg/mL, (e) 25 µg/mL, (f) 50 µg/mL, and (g) 100 µg/mL. (B) Graphical representation of the neuroprotective effect of the pigment assessed by MTT assay. All experiments were performed in triplicates, and results are expressed as mean ± SD. Data were analyzed using one-way ANOVA followed by Dunnett's test. ***$p < 0.001$, *$p < 0.01$ compared to the rotenone-exposed group.

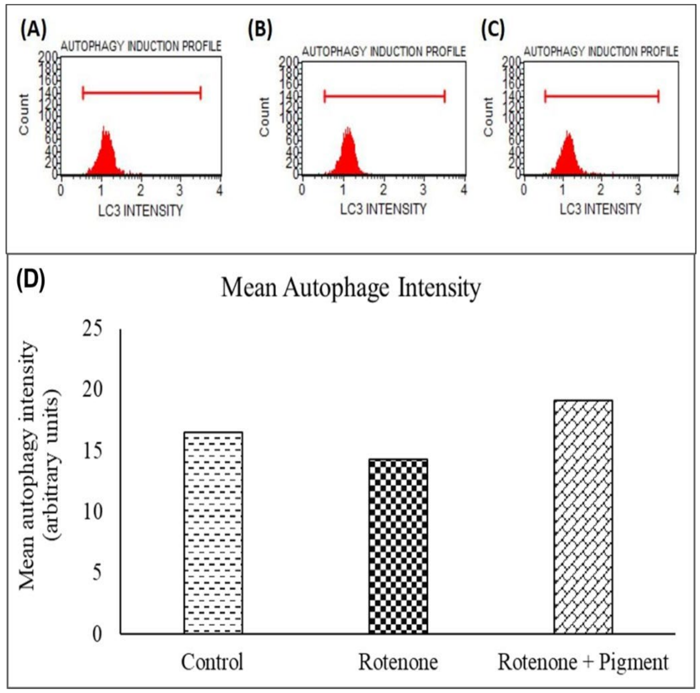

Figure 4: FACS analysis of autophagy activity of isolated pigment in rotenone-exposed SH-SY5Y cells. (A) untreated SH-SY5Y cells (B), rotenone-exposed SH-SY5Y cells (C) Rotenone-exposed cells co-administered with 25 μg/mL of pigment extracted from *M. luteus* (D) Graphical representation depicting the autophagy activity of isolated pigment in rotenone-exposed SH-SY5Y cells by FACS analysis.

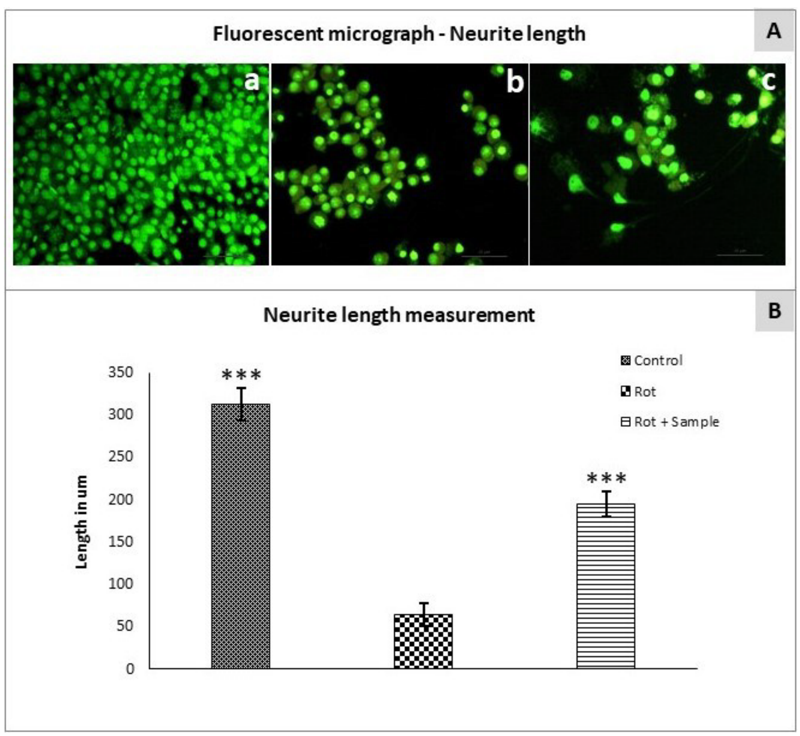

Figure 5. (A) Fluorescent micrographs (40X magnification) demonstrating neurite length in (a) untreated SH-SY5Y cells, (b) cells exposed to rotenone alone, and (c) cells exposed to rotenone co-administered with 25 μg/mL *M. luteus* pigment. (B) Neurite length measurements in untreated SH-SY5Y cells, cells exposed to rotenone alone, and cells exposed to rotenone co-administered with 25 μg/mL *M. luteus* pigment, with experiments performed in triplicates, results expressed as mean ± SD, and data analyzed using one-way ANOVA followed by Dunnett's test (***$p < 0.001$ compared to the rotenone-exposed group).

3.3 Autophagy assay

The flow cytometric analysis of autophagy is illustrated in Figure 4, with LC3 intensity serving as a marker that typically signifies the formation of autophagosomes. The LC3 fluorescence intensity distribution from cells stained for autophagy detection is depicted in each histogram. The baseline LC3 intensity exhibits a moderate peak within a defined range. Rotenone treatment exhibits a leftward shift and a narrower peak, signifying diminished LC3 intensity and consequently inhibited autophagy. In the rotenone + pigment treatment, the LC3 intensity peak shifts rightward, akin to the control, indicating a restoration or enhancement of autophagy. The findings indicate that the *M. luteus* pigment has the ability to stimulate autophagy, which could potentially offer neuroprotective benefits.

3.4 Neurite length

Control cells exhibit significant neurite outgrowth characterized by elongated processes and robust neuronal morphology. Cells treated with rotenone display significant neurite retraction and a rounded morphology, signifying neurotoxicity and cytoskeletal disintegration. Cells on treatment with rotenone and *M. luteus* pigment exhibited partially restored neurite outgrowth, evidenced by the elongation of processes, suggesting a protective influence on neurite architecture as shown in Figure 5A. Quantitative analysis revealed that neurite length was found to be significantly reduced (~75% reduction) after rotenone treatment when compared to the control group. The use of *M. luteus* pigment in conjunction with rotenone resulted in a statistically significant increase in neurite length, suggesting a partial restoration of the neuronal integrity, as shown in Figure 5B.

3.5 Analysis of ROS

The reactive oxygen species formed intracellularly in cells exposed to rotenone were observed and analyzed using fluorescence intensity by labeling the cells with DCFDA. Control cells display negligible green fluorescence, signifying low basal levels of ROS Cells treated with rotenone exhibit intense green fluorescence, indicating a significant elevation in ROS production resulting from mitochondrial dysfunction. Cells co-treated with rotenone and *M. luteus* pigment exhibit significantly diminished fluorescence, suggesting that the pigment has the capacity to mitigate rotenone-induced oxidative stress (Figs. 6A &B).

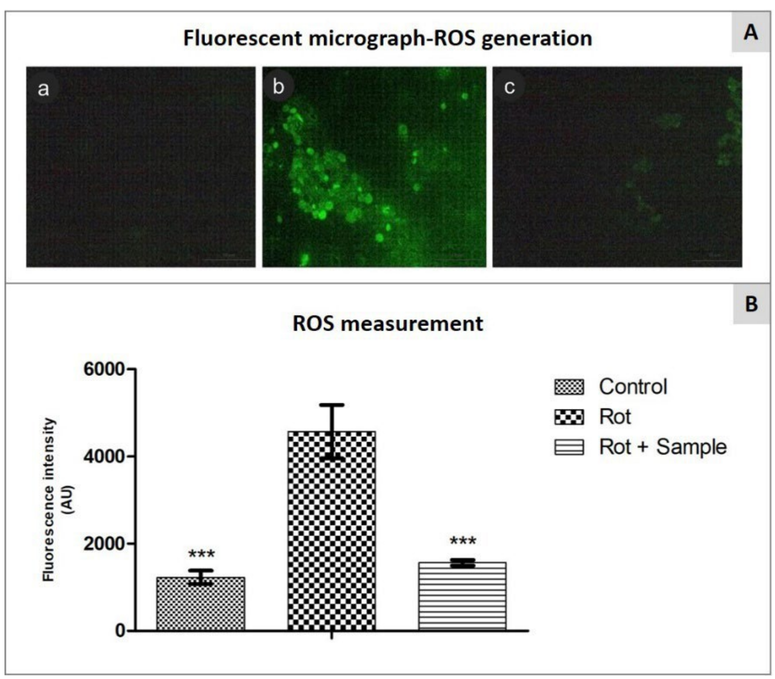

Figure 6. (A) Fluorescent micrographs (10X magnification) showing ROS generation in (a) untreated SH-SY5Y cells, (b) cells exposed to rotenone alone, and (c) cells exposed to rotenone co-administered with 25 µg/mL *M. luteus* pigment. (B) *In vitro* ROS measurement in untreated SH-SY5Y cells, cells exposed to rotenone alone, and cells exposed to rotenone co-administered with 25 µg/mL *M. luteus* pigment, with experiments performed in triplicates, results expressed as mean ± SD, and data analyzed using one-way ANOVA followed by Dunnett's test (***p < 0.001 compared to the rotenone-exposed group).

3.6 Effect of pigment on the expression of BDNF in SH-SY5Y cells

BDNF is a neurotrophin crucial for neuronal survival, development, and synaptic plasticity. Rotenone demonstrates significant downregulation of BDNF. This verifies that rotenone markedly inhibits BDNF expression, presumably as a result of oxidative stress, aligning with its neurotoxic characteristics. The neuroprotective and potentially neuro-regenerative capabilities of BDNF are indicated by the effective reinstatement and amplification of BDNF expression through co-treatment with *M. luteus* pigment (Figure 7).

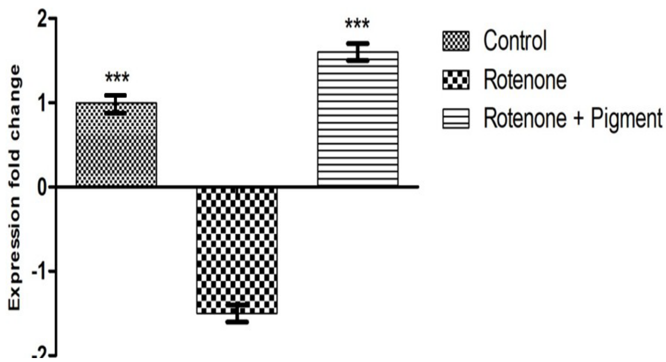

Figure 7: Graphical representation illustrating the BDNF expression by semi-quantitative real-time PCR analysis. Along X-axis: Samples; Along Y-axis: Expression fold change. All experiments were done in triplicates and results represented as mean ± SD. One-way ANOVA and Dunnetts tests were performed to analyze data. ***p< 0.001 compared to rotenone-exposed group.

4. DISCUSSION

Parkinson's disease is a common neurodegenerative condition predominantly affecting the geriatric population and ranks as the second leading cause of dementia. The progressive deterioration of dopaminergic neurons in the substantia nigra pars compacta represents the primary pathogenic change linked to the disease [22, 23]. Currently available PD drugs offer symptomatic relief, but they cannot stop the progression of the deteriorating process. Long-term usage of the drugs can lead to progressive decline in drug response, motor instability, and drug-prompted toxicity. Natural products that can provide neuroprotective support independently or in combination with the existing drugs can be an alternative therapeutic strategy [24]. In this study, we analyzed the effect of the pigment produced from *M. luteus* isolated from soil on rotenone-induced neurodegenerative changes in SH-SY5Y cell lines. A comparative study will be advantageous in recognizing the functional diversity among various species, thereby offering valuable insights into the strain-specific pigment biosynthesis pathways and functional variations. Futuristic research may benefit from this methodology. This advancement may facilitate a more comprehensive understanding of the biological characteristics of various species.

Column chromatography using a silica column was employed to isolate and purify the yellow pigment, which was subsequently characterized using FTIR. Peaks were observed at 3269.07 cm-1, 1629.79 cm-1, as well as 1015.55 cm-1, that is linked to distinct functional groups. The –OH stretching is indicated by a broad and robust peak that spans from 3700 to 3000 cm-1 [25].

Rotenone, an organic pesticide and a powerful inhibitor of mitochondrial complex I, induces characteristics that are indistinguishable from those observed in clinical PD. It causes deterioration of the dopaminergic system, neurodegeneration, neuroinflammation, and behavioral abnormalities [26]. The neuroprotective effect of the isolated pigment was investigated using a PD cellular model generated by rotenone.

Reactive oxygen species are highly active molecules responsible for the emergence of neurodegenerative diseases. Enhanced oxidative stress levels are often seen in the brains of individuals with neurodegenerative diseases [27]. Cell viability after co-treatment using rotenone and pigment was shown to be improved compared to rotenone treatment. Cell viability was enhanced by the pigment at a pace that was dependent on concentration. The MTT analysis confirmed that the pigment has neuroprotective properties. Results from measuring neurite length and ROS using DCFDA demonstrated that the bacterial pigment provided neuroprotection. Our findings were consistent with the results from previous investigations [28]. Studies suggest that β-carotene reduces ROS accumulation and protects neurons from oxidative damage. It enhances the expression of downstream antioxidant enzymes, contributing to its neuroprotective effects [29].

Autophagy is a crucial intracellular mechanism that removes dysfunctional structures and misfolded proteins to maintain cell homeostasis. When autophagic activity in neurons is either too high or too low, it disrupts balance and reduces neuron survival, leading to neurodegeneration. Rotenone has been observed to impede autophagic flow before inducing cell death [30]. Xiong *et al* have reported a decline in the expression of autophagic markers LC3 and adaptor protein autophagy P62 in rotenone-treated SH-SY5Y cells. Studies have revealed that enhancers of autophagy prevented the toxicity induced by rotenone through boosting autophagy in SH-SY5Y cells [31]. Induction of autophagy as a therapeutic approach for neurodegenerative disorders was reported by Djajadikerta A *et al* [32].

Muse Cell Analyzer flow cytometric analysis showed that autophagy, which had been suppressed by rotenone treatment, was reactivated by pigment derived from *M. luteus*. One common model for pathologies similar to PD is rotenone, which is known to cause neuronal damage via oxidative stress and impaired autophagic flux [33]. In this investigation, we found that rotenone exposure drastically decreased autophagic activity. It is worth noting that the *M. luteus* pigment revived autophagic activity after treatment, which raises the possibility that it modulates autophagy and acts as a neuroprotective agent. This recovery might be because the pigment, which is rich in carotenoids, has antioxidant qualities that reduce oxidative stress and restore normal autophagic flux. Studies have shown that natural antioxidants can counteract rotenone-induced autophagy inhibition by diminishing ROS accumulation and regulating critical pathways such as AMPK-mTOR signaling [34, 35]. Our results indicate that *M. luteus* pigment not only mitigates rotenone-induced oxidative injury but may also reinstate cellular homeostasis by reactivating autophagy.

BDNF, a key member of the neurotrophin growth factor family, supports the health and function of striatal neurons. It promotes their development, survival, and adaptability throughout life. A reduction in BDNF levels can lead to the deterioration of these neurons, causing symptoms resembling those of Huntington's disease, including movement, cognitive, and behavioral issues [36]. Studies have reported that low levels of BDNF in serum may contribute to the etiology of restless legs syndrome in PD, suggesting that it may be a valuable blood biomarker [37]. BDNF and its receptor signaling cascades regulate synaptic plasticity, which is crucial to memory and learning. Variations in signaling pathways and BDNF levels have also been reported in AD [38]. Due to its function in the formation and maintenance of the central and peripheral nervous systems, BDNF has been identified as a potential therapeutic target against PD. Individuals afflicted with PD exhibit reduced BDNF levels in their bloodstream [39].

Natural compounds like phytochemicals, found in plants, are noted for their neuroprotective properties, including their potential to influence BDNF levels and activity. Carotenoids such as lycopene, astaxanthin, and β-Carotene are lipophilic pigments recognized for their powerful antioxidant properties. They effectively neutralize ROS, protecting neuronal cells from oxidative damage, an important factor in the pathogenesis of neurodegenerative diseases. Lycopene administration has been linked to nerve growth factor production and higher BDNF during neurotoxic challenges [40]. Lycopene preserves mitochondrial function, antioxidant enzyme activity, and animal behavior. In preclinical PD models, lycopene exhibits stronger and consistent neuroprotection than β-carotene, despite both carotenoids exert radical scavenging and anti-inflammatory effects [41, 42]. Carotenoids upregulate BDNF expression and activate its downstream signaling pathways (PI3K/Akt and MAPK/ERK), promoting neuronal survival, synaptic plasticity, and cognitive function [43]. Fucoxanthin, a natural orange carotenoid, is also reported to enhance BDNF expression [44]. Direct antioxidant properties and neurotrophic support of carotenoids make them interesting candidates for treatment and prevention of neurodegenerative diseases. Investigating the clinical relevance of carotenoid-based treatments in neurodegenerative diseases and clarifying the exact molecular interactions involved is highly significant. So we are planning to pursue the study as a continuation of the present work. Our study established that the bacterial pigment treatment significantly stimulated BDNF mRNA expression in rotenone-treated cells. The neuroprotective effect of *M. luteus* pigment was also demonstrated in SH-SY5Y neuroblastoma cell lines that were exposed to rotenone, which was employed to create a Parkinson's disease model.

5. CONCLUSION

The carotenoid pigment isolated from *M. luteus* exhibits strong neuroprotective effects in rotenone-induced SH-SY5Y neuroblastoma cells by enhancing cell viability, reducing oxidative stress, regulating autophagy, and upregulating BDNF expression. These findings suggest that the pigment holds promise as a natural therapeutic candidate for the management of PD and related neurodegenerative disorders. But before going to the translational application, *in vivo* confirmation must be conducted. The evidence for the functional increase of BDNF is further undermined by the absence of protein-level confirmation of its expression by confirmatory tests like Western blotting or ELISA.

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Conflicts of interest

On behalf of all the authors, the corresponding author states that there are no conflicts of interest.

Data availability statement

All data generated or analyzed during this study are included in this published article. Additional datasets are available from the corresponding author on reasonable request.

Abbreviations

AD: Alzheimer's disease
BDNF: Brain-derived neurotrophic factor
CLIF: Central Laboratory for Instrumentation and Facilitation
DCFDA: Dichloro dihydro fluorescein diacetate
DMEM: Dulbecco's Modified Eagle's Medium
DMSO: Dimethyl sulphoxide
HCCA: α-cyano-4-hydroxycinnamic acid
MALDI-TOF MS: Matrix-assisted laser desorption ionization-time of flight mass spectrometry
MTT:3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCCS: National Centre for Cell Sciences
PBS: Phosphate buffered saline
PD: Parkinson's disease
RA: Retinoic acid

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