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C-phycocyanin shows neuroprotective effect against rotenone-induced Parkinson's disease in mice

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ABSTRACT

Objective: To investigate the neuroprotective effect of C-phycocyanin in a mouse model of rotenone-induced Parkinson's disease.

Methods: C-phycocyanin (50 mg/kg, *i.p.*, daily) was administered to rotenone (30 mg/kg, *p.o.*, daily) treated mice for 28 days. Behavioral studies (Y-maze, rotarod, round beam walk, and wire-hang tests) were carried out to assess neurobehavioral deficits. Glutathione and malondialdehyde were determined in both serum and striatal tissue. Molecular proteins (AKT, AMPK, NF- κ B, BDNF, and alpha-synuclein) in the striatum were estimated using ELISA. Histopathological analyses (hematoxylin and eosin staining as well as Nissl staining) were carried out to assess structural abnormalities in the striatum.

Results: C-phycocyanin significantly increased BDNF levels and decreased alpha-synuclein levels. It also slightly upregulated AMPK and AKT levels without significant difference compared with the rotenone group. Additionally, rotenone-induced elevated oxidative stress and structural abnormalities in the striatum were markedly mitigated by C-phycocyanin.

Conclusions: C-phycocyanin might have potential neuroprotective effects against Parkinson's disease. Further studies are warranted to verify its efficacy and to understand the molecular mechanisms behind the neuroprotective effects of C-phycocyanin in Parkinson's disease.

KEYWORDS: Parkinson's disease; C-phycocyanin; Alpha-synuclein; BDNF; Oxidative stress; Rotenone

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative

disorder that affects substantia nigra pars compacta (SNpc) region of the brain and ultimately leads to dopaminergic (DA) insufficiency[1]. It is most prevalent in elderly people. PD affects around 1% of people above age 60 years and around 7-8 million people across the world[2]. Clinical manifestations of the disease include both motor and non-motor symptoms. Non-motor symptoms like olfactory dysfunction and gastrointestinal dysfunction are present in the early stages of PD, but they are not distinctive[3]. The motor symptoms including dyskinesia, tremors, bradycardia rigidity, and impaired gait are visible after substantial neuronal loss. Current therapies for PD majorly involve symptomatic treatment, including levodopa, monoamine oxidase-B inhibitors, DA facilitators, and catechol-*O*-methyl-transferase inhibitors amongst others[4,5]. However, there is still a lack of therapies that affect disease progression.

Significance

Parkinson's disease is a neurodegenerative disorder and leads to dopaminergic deficiency. The progressive disorder mainly occurs due to aberrant alpha-synuclein and its aggregation. In order to halt the progression of this disease, therapies targeting alpha-synuclein need to be formulated. This study demonstrates that C-phycocyanin exerts neuroprotective effects against rotenone-induced Parkinson's disease by modulating BDNF and alpha-synuclein levels, decreasing oxidative stress, and mitigating structural changes in the striatum.

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Alpha-synuclein aggregation is believed to be the major cause behind the pathology. These aggregates form Lewy bodies and cause degeneration of DA neurons in the SNpc and striatum[6]. The physiological role of alpha-synuclein includes vesicle recycling, vesicular trafficking, and lipid interaction and it is also actively involved in the formation of SNARE assembly[7]. However, upon its aberration, it may undergo point mutations. Phosphorylation of alpha-synuclein at ser129 position leads to aggregation[8]. A protein glycogen synthase kinase-3 β (GSK-3 β) promotes hyperphosphorylation and thus may lead to increased aggregation and cellular build-up of alpha-synuclein. Moreover, GSK-3 β activation is implicated in neuroinflammatory processes and oxidative stress[9–12]. Thus, inhibiting GSK-3 β may be helpful for reducing alpha-synuclein aggregation. Upregulation of phosphoinositide-3 kinase/protein kinase B (PI3K/AKT) is responsible for downregulating GSK-3 β [9,13–17]. Thus, PI3K/AKT activation may hinder alpha-synuclein aggregation and accumulation. Downstream signaling leads to activation of brain-derived neurotrophic factor (BDNF) *via* cAMP response element binding protein (CREB) and it renders neuroprotection. Furthermore, there are other pathological features associated with PD pathology, including neuroinflammation, impaired autophagy, mitochondrial dysfunction, and oxidative stress. GSK-3 β facilitates NF- κ B mediated neuroinflammation. Thus, this can be avoided by its inhibition. AMPK activation can enhance autophagy and restore its functioning[18,19]. Moreover, GSK-3 β is also implicated in dysfunctional mitochondria and impaired mitophagy[10–12,20]. Thus, all these pathological features are associated with GSK-3 β in some way and can be alleviated by the means of its inhibition. Conclusively, PI3K/AKT activation strategy may render neuroprotection and halt the spread of PD pathology.

C-phycocyanin is a bluish-coloured protein obtained from green algae *Spirulina platensis* with antioxidant and neuroprotective properties, which has been established in a 6-OHDA model of PD[21]. It is known to modulate the PI3K pathway and AMPK pathway. This pathway further leads to activation of other downstream signaling. C-phycocyanin has neuroprotective potential, however, the precise mechanism behind its actions remains unclear. Hence, in this study, we aimed to evaluate its effect in a mouse model of rotenone-induced PD.

2. Materials and methods

2.1. Chemicals and reagents

C-phycocyanin was obtained from the AUM Research Laboratories, Rakanpur, Ahmedabad, Gujarat, India as a gift sample. Rotenone was obtained from TCI Chemicals (India) Pvt Ltd. ELISA kits required for estimation of molecular proteins were acquired from

Krishgen Biosystems, India. All the chemicals utilized in this study were of analytical grade.

2.2. Experimental animals

ICR mice were procured from Zydus Research Centre, Ahmedabad, India. The animals weighing 28–30 g were housed at $(25 \pm 2)^\circ\text{C}$ with 12 hours of light and dark cycles and 45% to 65% relative humidity throughout the research period at the Institute of Pharmacy, Nirma University. They were given standard laboratory food and drinking water *ad libitum*.

2.3. Experimental protocol

All the animals were allowed to acclimatize to the housing facility for 7 d before initiation of the protocol. The experiment protocol lasted for 28 d. Thirty-two male ICR mice were assigned to four groups ($n=8$). Group 1: Control, animals received 0.5% *w/v* carboxymethylcellulose (CMC) in water (0.5 g CMC in 100 mL water); Group 2: Rotenone, animals were given 30 mg/kg rotenone *p.o.* daily for 28 d; Group 3: C-phycocyanin, animals received 50 mg/kg C-phycocyanin *i.p.* for 28 d, daily; Group 4: Rotenone + C-phycocyanin, animals were given both 30 mg/kg rotenone *p.o.*[22,23] and 50 mg/kg C-phycocyanin *i.p.* daily for 28 d.

Rotenone is insoluble in water. Thus, the stock solution (50 mg/kg rotenone) was prepared using chloroform. This solution was stored at -20°C for 2 weeks. For induction, a rotenone working solution was prepared from the stock in CMC (0.5%). For C-phycocyanin, sterile water was used as a vehicle. After 1 h of rotenone administration, C-phycocyanin treatment was given to the animals. Dose as well as route of administration of rotenone and C-phycocyanin were decided based on previously conducted studies[24,25]. Behavioral assessments were carried out every alternate week.

After the completion of study, serum was collected and animals were euthanized. Whole brain tissues were collected from half of the animals and subjected to 10% formalin for fixation for further histopathological studies. From the remaining animals, striatum tissue was isolated and stored at -20°C for biochemical and molecular protein estimations. Experimental design is shown in Figure 1.

2.4. Neurobehavioral assessments

Four different neurobehavioral assessments were carried out that included: 1) Rotarod test (for determination of motor coordination), 2) Beam walk test (for motor dysfunction), 3) Wire-hang test (for determination of muscle strength), and 4) Y-maze (to assess cognitive impairment). Behavioral studies were conducted at 0 week, and at the end of the 2nd and 4th weeks. Before each behavioral assessment, animals were acclimatized in the behavioral room for

1 h. After every trial, the apparatus used for behavioral studies was cleaned with 5% acetic acid. The rotarod test was performed to determine motor coordination and function. The mice were placed on the cylinder in the rotarod apparatus. The latency to fall (s) was recorded. The trial was conducted for 5 min. During the test duration, speed escalated from 10 rpm to 20 rpm. The mean duration from 3 consecutive trials was recorded. There was a 30-minute interval between consecutive trials[26]. The round beam walk test was performed to determine motor dysfunction and coordination. Two different round beams with 25 mm and 15 mm diameter were taken for this test. Initially, training to cross the beam was given to animals using the wider beam. For the final trial, a narrow beam was used. The time required for the mice to cross the beam and the number of slips by the mice during the beam walk were recorded. The wire-hang test was carried out to assess the muscle strength of the animals. Mice were suspended from a metallic wire at 3 ft height above ground attached to 2 poles. A home cage was placed below the suspended wire. Latency for the animal to lose the grip and fall was recorded. Mean latency was determined from the average of 3 trials. Every trial was carried out after 30 min intervals[26]. The Y-maze test was used to assess degree of cognitive impairment. A Y-shaped apparatus was used. The three arms of the apparatus had been labeled as A, B and C. Mice were positioned in the center of the Y-maze apparatus. For around 7 min, the animal was allowed to explore the Y-maze. The path covered by mice was determined and % spontaneous alternations were calculated. The formula for calculation of % spontaneous alternations is as follows: $[\text{number of alternations (linear arm entries)} / (\text{total number of arm visits} - 2)] \times 100$ [9,27,28].

2.5. Enzyme-linked immunosorbent assay (ELISA)

Striatum tissue lysate was prepared using radioimmunoprecipitation (RIPA) lysis buffer. The tissue was subjected to sonication cycles and centrifugation ($12000 \times g$ for 7 min at 4°C) after addition of RIPA buffer. After that, the supernatant was collected and stored at -20°C . For ELISA, tissue lysate was used as a sample. Levels of molecular proteins [AKT (S473, KLR1201), AMPK (Thr172, KLM2345), BDNF (KLM0013), NF- κ B (KLR0287) and alpha-synuclein (pathological form, KLM1401)] were determined using ELISA Kits (Krishgen

Biosystems, Mumbai, India). Experimental procedure was as per the standard protocol provided by per manufacturer.

2.6. Oxidative stress assays

Tissue lysate was prepared as described in the previous section. Reduced glutathione (GSH) is an oxidative stress marker. In PD pathology, an antioxidant-oxidant balance is disrupted and hence oxidative stress rises. Tissue lysate/serum (100 μL) was added to 400 μL phosphate buffer (pH 7.4) and 1500 μL 5,5-dithiobis-2-nitrobenzoic acid. This mixture was incubated at 50°C for 1 h. Pale yellow coloration developed, and intensity was measured at 412 nm. The unknown concentrations of GSH were determined from the standard curve[9].

Lipid peroxidation process is an indicator of oxidative stress in the body and malondialdehyde (MDA) is the end product of this process. MDA levels were determined using thiobarbituric acid (TBA) assay. Then 100 μL sample was added to a mixture of 100 μL sodium dodecyl sulfate and 750 μL TBA. It was then subjected to incubation at 95°C for 90 min till color change was observed. Intensity was measured at 532 nm and an unknown concentration was determined using a standard curve[9].

2.7. Histopathological analysis

Brain tissues were subjected to treatment with 10% formalin for fixation. They were then embedded in paraffin. The sections were cut at 5 μm thickness. Thereafter, the slides were stained with hematoxylin and eosin (H & E) as well as Nissl stain for examining structural alterations. Striatal area was visualized at low power ($10\times$ magnification) using a light microscope (Olympus CKX41, Japan). In Nissl-stained sections, neuronal counting was done manually using ImageJ software.

2.8. Statistical analysis

Data are reported as mean $\pm$ SEM using GraphPad V8. One-way ANOVA followed by Bonferroni's *post hoc* test was applied. $P < 0.05$ was considered statistically significant.

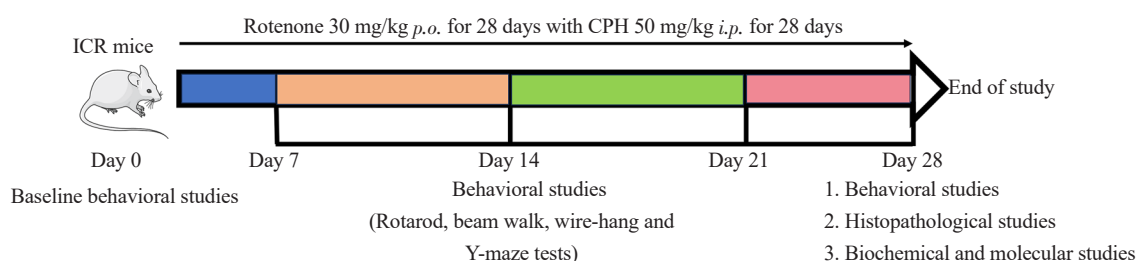


Figure 1. Experimental design. CPH: C-phycoerythrin.

2.9. Ethical statement

The experimental protocol was approved by Institutional Animal Ethics Committee of Institute of Pharmacy, Nirma University, Ahmedabad in accordance with the Committee for Control and Supervision of Experiments on Animals of the Ministry of Social Justice and Empowerment of the Government of India with a protocol number: IP/PCOL/MPH/32/2022/11.

3. Results

3.1. Effect of C-phycocyanin on behavioral deficits induced by rotenone administration

After 28 days of rotenone exposure, it was observed that there was a significant decrease ($P<0.01$) in latency to fall as compared to the control in wire-hang test, indicating loss of muscle strength (Figure 2A). Administration of C-phycocyanin in rotenone-induced PD animals led to increased latency, however, it was insignificant.

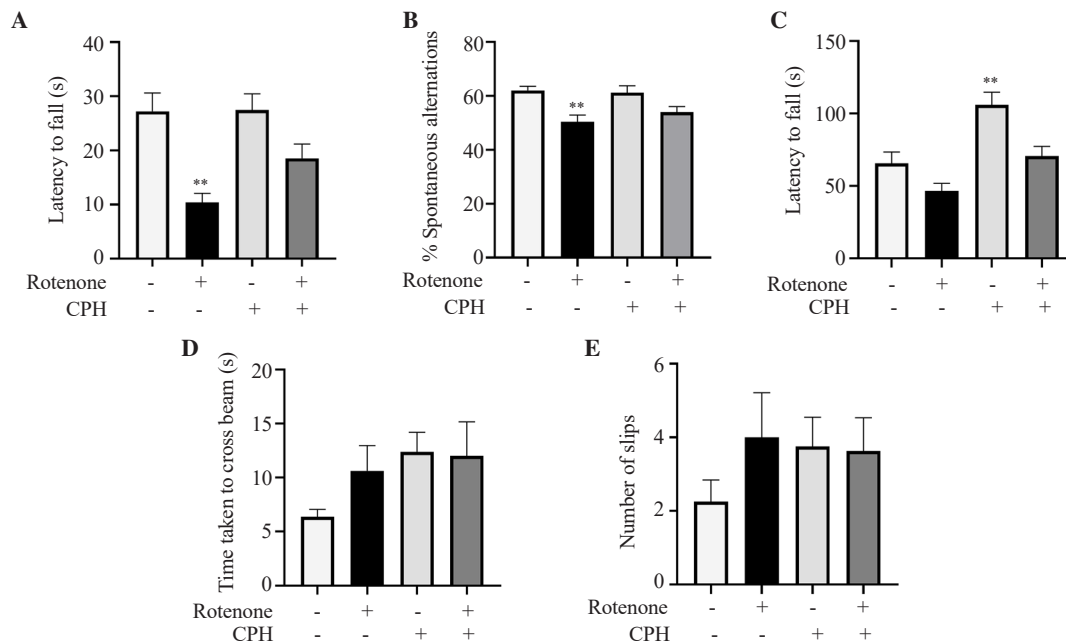


Figure 2. Effect of C-phycocyanin treatment on behavioral parameters in mice with rotenone-induced Parkinson's disease. (A) Wire-hang test, (B) Y-maze, (C) rotarod, (D-E) round beam walk. Data is presented as mean $\pm$ SEM ($n=8$) and analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. ** $P<0.01$ vs. the control group.

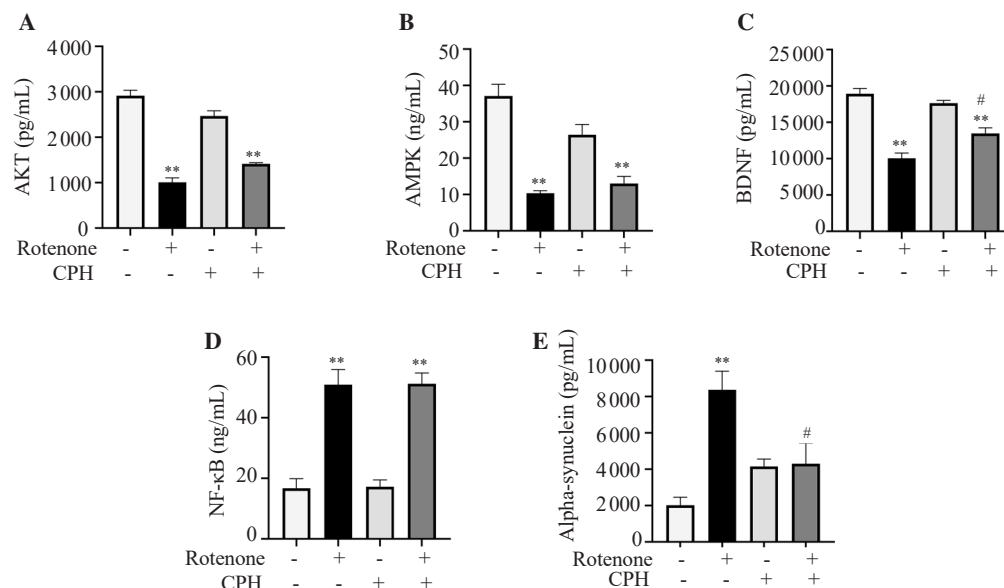


Figure 3. Effect of C-phycocyanin treatment on protein expression of (A) AKT, (B) AMPK, (C) BDNF, (D) NF-κB, and (E) alpha-synuclein in mice with rotenone-induced Parkinson's disease. Data is presented as mean $\pm$ SEM ($n=3$) and analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. ** $P<0.01$ vs. the control group; # $P<0.05$ vs. the rotenone group.

The control and C-phycoerythrin alone groups showed almost similar latency to fall in this test (Figure 2A). Significantly ($P<0.01$) fewer spontaneous alternations were observed in the rotenone group at the end of study as compared to the control in Y-maze test, revealing cognitive deficit due to rotenone exposure. Mice given rotenone and C-phycoerythrin administration showed slightly higher % spontaneous alternations and increased latency compared with the rotenone group (Figure 2B and C). In addition, the C-phycoerythrin alone group surprisingly showed significantly ($P<0.01$) higher latency than control animals in rotarod test (Figure 2C). There was

no significant change in the number of slips and the time required to cross the beam in the beam walk test amongst all groups. However, it could be seen that the time for crossing the beam was higher in all treatment groups than in the control group (Figure 2D). It was also evident that the number of slips was higher in the rotenone-treated animals than in the control and other treatment groups (Figure 2E). Nevertheless, there was no significant difference between all groups in the beam walk test (Figure 2D and E). The results indicate that C-phycoerythrin administration in rotenone-induced PD mice could alleviate rotenone-caused behavioral deficits to some extent,

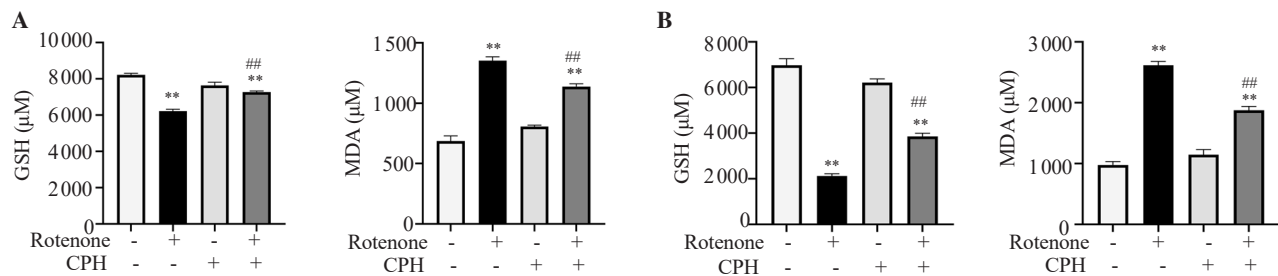


Figure 4. Effect of C-phycoerythrin treatment on oxidative stress markers in (A) striatum tissue and (B) serum of mice with rotenone-induced Parkinson's disease. Data is presented as mean $\pm$ SEM ($n=3$) and analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. ** $P<0.01$ vs. the control group; ## $P<0.01$ vs. the rotenone group.

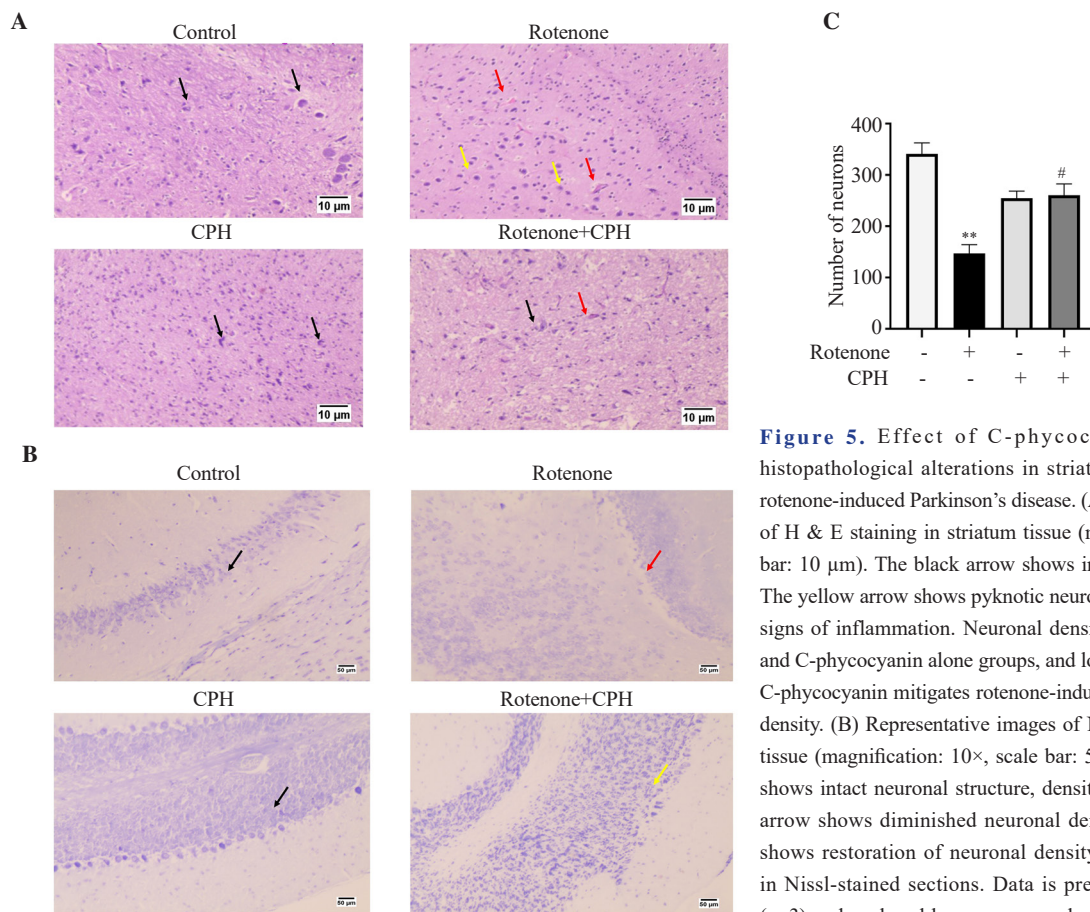


Figure 5. Effect of C-phycoerythrin treatment on histopathological alterations in striatum tissue of mice with rotenone-induced Parkinson's disease. (A) Representative images of H & E staining in striatum tissue (magnification: $10\times$, scale bar: $10\mu\text{m}$). The black arrow shows intact neuronal structures. The yellow arrow shows pyknotic neurons. The red arrow shows signs of inflammation. Neuronal density is high in the control and C-phycoerythrin alone groups, and low in the rotenone group. C-phycoerythrin mitigates rotenone-induced decrease in neuronal density. (B) Representative images of Nissl staining in striatum tissue (magnification: $10\times$, scale bar: $50\mu\text{m}$). The black arrow shows intact neuronal structure, density, and integrity. The red arrow shows diminished neuronal density. The yellow arrow shows restoration of neuronal density. (C) Neuronal number in Nissl-stained sections. Data is presented as mean $\pm$ SEM ($n=3$) and analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. ** $P<0.01$ vs. the control group; # $P<0.05$ vs. the rotenone group.

however, the difference in behavioral parameters between the group administered with C-phycoerythrin and rotenone and the rotenone alone group was insignificant (Figure 2).

3.2. Effect of C-phycoerythrin on protein levels

The expressions of AKT, AMPK, and BDNF were significantly reduced ($P<0.01$) in the rotenone-administered group as compared to the control group. However, C-phycoerythrin treatment upregulated the expression of AKT and AMPK with an insignificant difference compared with the rotenone-administered group (Figure 3A-B). BDNF expression was significantly increased by C-phycoerythrin treatment ($P<0.05$) (Figure 3C). Moreover, rotenone administration markedly increased NF- κ B and alpha-synuclein expression ($P<0.01$) compared with the control group. Treatment with C-phycoerythrin markedly reduced alpha-synuclein expression ($P<0.05$) while inducing no significant changes in NF- κ B expression ($P>0.05$) (Figure 3D-E). The C-phycoerythrin alone group showed no noticeable changes in these proteins compared with the control group ($P>0.05$).

3.3. Effect of C-phycoerythrin on oxidative stress markers

In striatum tissue and serum, GSH level was significantly decreased and MDA level increased ($P<0.01$) in the rotenone group compared with the control group animals (Figure 4). Treatment with C-phycoerythrin could mitigate rotenone-induced alterations in GSH

and MDA levels ($P<0.01$). Additionally, there was no significant difference in MDA and GSH levels between the C-phycoerythrin alone treated group and the control group in both serum as well as striatal samples ($P>0.05$).

3.4. Effect of C-phycoerythrin on histopathological alterations induced by rotenone exposure

In H & E-stained sections, it was observed that intact neuronal structures were present in the control and C-phycoerythrin alone groups. In the rotenone group, obvious signs of inflammation and pyknotic neurons were seen. In contrast, treatment with C-phycoerythrin after rotenone exposure showed restored neuronal density as well as intact neuronal structures (Figure 5A). In Nissl-stained sections, it was found that the control and C-phycoerythrin alone groups had high neuronal density as compared to the rotenone group. In the rotenone group, neuronal density was decreased in Nissl-stained sections, which was restored by C-phycoerythrin treatment (Figure 5B). In addition, as shown in Figure 5C, neuronal number was significantly lower ($P<0.01$) in the rotenone group, which was markedly increased ($P<0.05$) by C-phycoerythrin treatment.

4. Discussion

C-phycoerythrin is a proteinaceous molecule that possesses

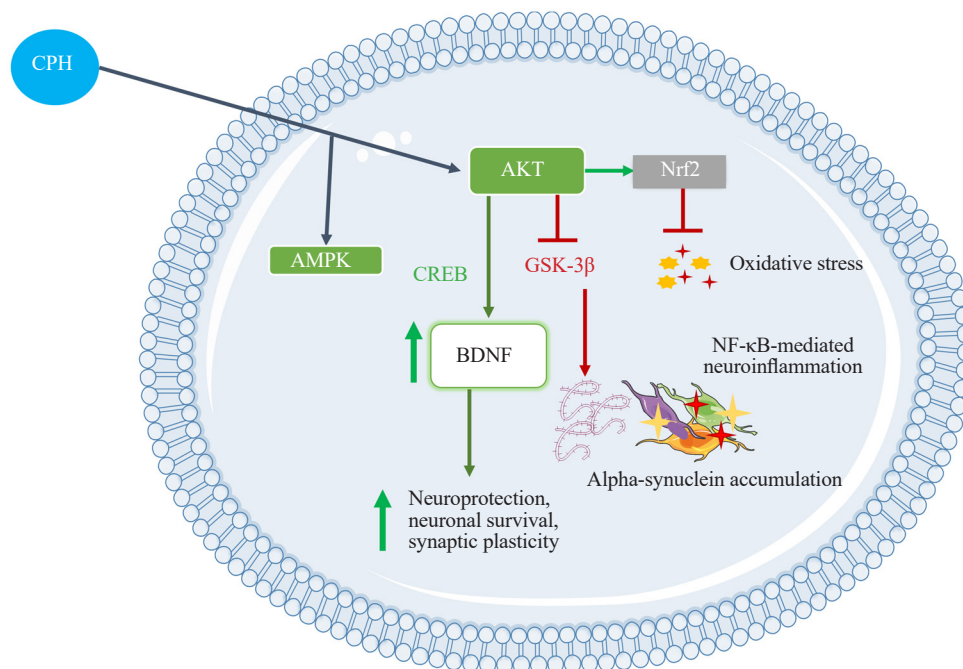


Figure 6. Possible molecular mechanism and rationale of the study.

antioxidant as well as neuroprotective potential. PD is a neurodegenerative disorder leading to DA deficiency and motor disabilities[1,9,29]. Alpha-synuclein aggregation, oxidative stress, neuroinflammation, mitochondrial dysfunction, and impairment of cellular clearance system such as autophagy are some of the prominent pathological features of PD[1,30]. It is now known that the BDNF and PI3K/AKT pathway is actively involved in modulation of PD pathogenesis[13–15]. Moreover, upregulation of AMPK signaling facilitates autophagy[18]. AKT and AMPK activation are suggested to slow down progression of PD pathology by targeting alpha-synuclein aggregation. Also, AKT activation might influence NF- κ B mediated neuroinflammation. The current study tried to explore neuroprotective activity of C-phycocyanin as well as elucidate the mechanism underlying its neuroprotective actions in PD.

C-phycocyanin is reported to activate the PI3K/AKT and AMPK signaling cascade *in vitro* in insulin-resistant HepG2 cells[24,31]. PI3K/AKT pathway upon activation is known to interrupt GSK-3 β signaling[17], thereby subsequently inhibiting hyperphosphorylation and aggregation of alpha-synuclein protein. GSK-3 β inhibition also alleviates NF- κ B mediated neuroinflammation. NF- κ B when activated leads to release of inflammatory cytokines that include IL-1 β , IL-6, and TNF- α . These further trigger apoptosis and neuronal death. Activation of NF- κ B is essential for these processes and might be influenced by alpha-synuclein aggregation[32,33]. AKT activation might ultimately lead to upregulation of BDNF. This leads to enhanced neuronal survival and synaptic plasticity. Moreover, AMPK activation may enhance autophagy and associated functions that are usually disrupted in pathological state of PD. Activation of the Nrf2/ARE pathway upregulates the synthesis of antioxidant proteins and thus alleviates oxidative stress (Figure 6). Oxidative damage plays a critical role in PD pathogenesis. In this study, we observed that chronic rotenone exposure for 28 d could induce oxidative stress in animals in the striatum.

In this study, C-phycocyanin (50 mg/kg *i.p.*, daily.) was administered along with rotenone (30 mg/kg *p.o.*, daily) for 28 d in ICR mice. Rotenone is a neurotoxin and it induces PD-like conditions[9]. Behavioral assessments were performed at the end of the second and fourth weeks. After the completion of the study, animals were euthanized and levels of BDNF, AKT, AMPK, NF- κ B, and alpha-synuclein were measured. Furthermore, levels of GSH and MDA were determined as a measure of oxidative stress in the striatum and serum. Neuronal structure in striatal tissue and SNpc is altered in pathogenesis of PD. H & E staining and Nissl staining have been performed to visualize such alterations in the striatal tissue in PD.

From the neurobehavioral assessments, it was established that rotenone induces behavioral deficits in mice after chronic exposure to Y-maze and wire-hang test. No significant changes were observed in rotarod and beam walk tests. In a study, it was reported that

Spirulina has a significant effect on behavioral parameters such as learning and motor functions in rodents[21]. Administration of C-phycocyanin restored behavioral functions only to a certain extent but without significant differences as compared to the rotenone-exposed group. A study reported that C-phycocyanin could upregulate BDNF, nerve growth factor, superoxide dismutase, and catalase while downregulating inflammatory factors in *in vitro* 2D astrocyte model and *in vivo* 3D tissue astrocyte model[34]. In another study, it was observed that *Spirulina* activated BDNF and thereby prevented amyloid beta-induced toxicity in PC12 cells *in vitro*[35]. These findings confirm that C-phycocyanin exerts a modulatory effect on BDNF levels. Consistent with these findings, in our study, C-phycocyanin significantly upregulates BDNF and downregulates alpha-synuclein in striatal tissue. Thus, by upregulation of BDNF, we can infer that it might render neuroprotection, increase neuronal survival, and enhance synaptic plasticity. It is revealed that upregulation of BDNF may also lead to CREB activation, which promotes transcription of neurotrophic factors and enhances neuroprotection[36]. Downregulation of alpha-synuclein levels indicates that pathological progression was halted. C-phycocyanin also modulated the levels of AKT, AMPK, and NF- κ B[24,31,34,37]. In a previous study, C-phycocyanin activated insulin receptor signaling/PI3K/AKT and AMPK signaling in hepatocytes. In striatal tissues, C-phycocyanin plays a modulatory role in AKT and AMPK levels, however, the effect was not significant. C-phycocyanin induced no changes in NF- κ B levels in rotenone-administered mice. C-phycocyanin is also well known for its antioxidant potential[34]. It is reported that *Spirulina* could elevate GSH levels and reduce MDA levels in the brain (cerebellum) of aged rats[36]. The results of the present study corroborated the antioxidant properties of C-phycocyanin. There was a significant increase in GSH level and a decrease in MDA level after administration of C-phycocyanin in the striatum and serum of rotenone-exposed mice. Reduction of oxidative stress indicates that C-phycocyanin has effective antioxidant abilities. Oxidative stress is largely implicated in PD pathogenesis. It can accelerate neurodegeneration and cell death, and impair cellular function, which paves the way for advancement of PD pathology[38]. Hence, alleviation of oxidative stress might aid in alleviating PD development. Histopathological studies also demonstrated that rotenone administration resulted in abnormalities in neuronal structure, which were reversed after the administration of C-phycocyanin. Furthermore, neuronal density and number were significantly increased as compared to the rotenone group, indicating restoration of neuronal loss in the striatum.

Despite the significant modulation of molecular parameters such as BDNF and alpha-synuclein, this study has several limitations that must be addressed. Firstly, the insignificant modulation of behavioral parameters, including the rotarod and beam walk tests, hinders our ability to fully assess the restoration of motor deficits. Additionally,

the levels of key signaling molecules such as AMPK, AKT, and NF- κ B were not significantly modulated, preventing us from establishing a definitive mechanism of action for C-phycocyanin. Moreover, the dosage and duration of the study may have influenced the outcomes. A higher dose of C-phycocyanin might be necessary to elucidate the mechanisms more clearly, and extending the study duration could potentially result in significant modulation of these molecular parameters. Furthermore, the study did not include assessments of phosphorylated forms of proteins, which might be crucial for understanding the detailed mechanisms underlying the observed effects. Future research should address these limitations by employing higher doses of C-phycocyanin, extending the study duration, and including comprehensive molecular analyses to confirm the exact mechanisms of C-phycocyanin in PD. These steps will provide a more robust understanding of the neuroprotective potential of C-phycocyanin and its applicability in therapeutic settings.

This study demonstrated that C-phycocyanin treatment upregulated BDNF levels as well as downregulated alpha-synuclein levels. Moreover, it restored neuronal density and demonstrated a prominent antioxidant effect in striatal tissue. Further, there was a slight improvement in behavioral parameters after administration of C-phycocyanin. It also demonstrated insignificant improvement in AKT, AMPK, and NF- κ B levels unlike those reported by previous studies. Additionally, C-phycocyanin might also be acting on other signaling cascades associated with BDNF and alpha-synuclein like CREB and GSK-3 β . However, further studies are warranted to gather more evidence in order to establish a precise mechanism behind the effects of C-phycocyanin.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

RS, JS, and HR contributed to idea of the manuscript. HR performed literature search. HR and RS performed the experiments. RS and HR wrote this manuscript. JS supervised the project and critically reviewed the manuscript.

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