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Sargassum wightii ameliorates anxiety-like behaviour and cognitive deficits in rotenone-induced parkinsonian rats

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ABSTRACT

Introduction: Parkinson's disease (PD) is a neurodegenerative disorder, basically manifested by motor symptoms. However, there are other associated non-motor features in PD, including depression, anxiety, and cognitive impairments that significantly affect the quality of life. Scientific reports have shown that *Sargassum wightii*, a brown seaweed, protects against rotenone-induced motor deficits, mitochondrial dysfunction, and oxidative stress in rats. We therefore, undertook this study to evaluate its efficacy in alleviating rotenone-induced non-motor symptoms such as anxiety-like behavior and cognitive deficits in rats.

Methods: Rotenone at a dose of 10 mg/kg was given orally for 28 days to induce PD model in male rats. The vehicle and the test drug were given orally daily, 1 hour prior to the rotenone administration. The protective effect of *S. wightii* (methanol extract at 400 mg/kg dosage) was assessed through an array of tests: Elevated plus maze test, Morris water maze test, and novel object recognition test. On the 28th day, the rats were sacrificed, and hippocampal neurobiochemical analyses were performed using high-performance liquid chromatography (HPLC).

Results: Co-administration of *S. wightii* reversed the rotenone-induced anxiety-like behavior and cognitive deficits to a significant extent ($P<0.001$). It also restored the hippocampal neurotransmitters (5-hydroxytryptamine, dopamine, and 5-hydroxy indole acetic acid) significantly ($P<0.001$).

Conclusion: *Sargassum wightii* provides neuroprotective effects and reduces the non-motor symptoms of PD. Therefore, it might be a novel insight into PD therapy.

Implication for health policy/practice/research/medical education:
The present research explains the scientific basis of neuroprotective activity of *Sargassum wightii* against the non-motor symptoms of PD, such as anxiety-like behavior and cognitive deficits. Therefore, this extract can be a potential candidate in herbal formulations as a neuroprotectant against PD.

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Introduction

Parkinson's disease (PD) is a common neurodegenerative disease affecting the elderly; the prevalence of which occupies the second position next to that of Alzheimer's disease. The cardinal manifestations of PD include a progressive decline in motor, cognitive, behavioral, and autonomic functions (1). Until now, the pharmacotherapy of PD has not been satisfactory because of its complex multifactorial character underlying its pathology and lifelong treatment. Therefore, recently, the bioactive compounds from plant sources are of a lot of interest as they are expected to have fewer side effects, even in the long-term use in therapy for PD. Rotenone, a pesticide, induces PD in rodents, which is a well-documented animal model. Attenuation of brain monoamines, such as 5-hydroxytryptamine (5-HT), dopamine, and nor-adrenaline, as well as the oxidative stress and neuroinflammation induced by rotenone, are the factors attributing to the non-motor and motor deficits associated with PD (2,3).

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Sargassum wightii, a brown seaweed, has been shown to possess anti-inflammatory, anti-oxidant, neuroprotective, and anticancer properties, as evidenced by several scientific reports (4,5). Therefore, it has gained much attention from the Agri-food industries. In one of our earlier researches, we established its protective effect against motor deficits induced by rotenone in parkinsonian rats. Despite the availability of evidence on the neuroprotective effect of *S. wightii* in various animal models, its effects on anxiety-like behavior and cognitive deficits in PD have not yet been explored. Addressing this subject, we carried out the present investigation to evaluate the protective role of *S. wightii* against anxiety-like behavior, cognitive impairments, and neurobiochemical deficits in PD in rats induced by rotenone.

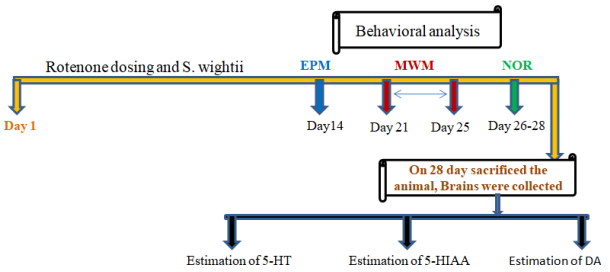

Figure 1. The timeline depicting the administration of drugs, behavioural assessments, and neurobiochemical analyses. EPM: elevated plus maze; MWM: Morris water maze; NOR: novel object recognition; DA: Dopamine; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid.

Materials and Methods

Animals

For this experiment, Male albino rats (150-200 g) of Wistar strain were used. Under standard laboratory environment, they were housed at 24 ± 1°C temperature, with 45-55% relative humidity, and a pattern of 12 hours light/dark cycle was maintained. They were allowed to regular food and drink conditions *ad libitum*. Upon obtaining the Institutional Animal Ethics Committee approval, the experiment was conducted as per CPCSEA guidelines.

Plant material

The dry powder of *S. wightii* was provided by Microbiotech Limited, Gujarat, India, as a gift sample. A known weight of *S. wightii* powder was subjected to a 72-hour Soxhlet extraction in methanol. The obtained mass was further dried using an evaporator and stored at 4°C for all experimental purposes.

Drugs and chemicals

Rotenone, dopamine hydrochloride, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were purchased from Sigma Aldrich, Pvt. Ltd. Octyl-sodium sulfate, EDTA, and sodium metabisulfite were from Hi-Media Laboratories Pvt. Ltd.

As detected in our previous experiments (6), polysaccharides, polyphenols, flavonoids, tannins, terpenoids, etc. were the important phytoconstituents of the methanol extract of *S. wightii.*

Acute toxicity test

For the acute toxicity study, following OECD Guidelines 423 [Limit test], the *S. wightii* extract was given orally, with 5, 50, 1000, and 2000 mg/kg doses. No change in behavior or mortality was observed within 24 hours of administration (7).

Experimental framework

Three groups of animals (n = 6 in each) were randomly assigned to receive various treatments orally for four weeks. Group I: Normal control (olive oil, 1 mL/kg), group II: Disease control (rotenone, 10 mg/kg orally) (8), group III: *S. wightii*, 400 mg/kg and rotenone, 10 mg/kg. One hour prior to rotenone, all the drugs were administered daily. From 14th day of treatment, the animals were subjected to behavioral tests. On the 28th day, all rats were euthanized by decapitation after assessing the behavioural performances, and the hippocampus was immediately dissected out (Figure 1).

Behavioral assessments

Elevated plus maze test

The method of Gopal Krishna et al was followed for assessing the anxiety using the elevated plus maze. The apparatus consisted of a wooden structure, raised to a height of 50 cm from the floor. It was made up of two open and two closed arms (50 × 10 cm) with a central platform of 10 × 10 cm size. Facing towards the open arm, each rat was placed on the central platform and allowed to explore it for five minutes. The time spent in open and closed arms measured the index of anxiety-like behaviour, and the number of entries indicated the locomotor activity (9).

Morris water maze test

The apparatus was a circular, water-filled pool divided into four equal quadrants with a submerged platform (10 × 10 cm) kept 2 cm below the water level. By randomly placing the rats in any of the four quadrants, they were initially trained for 2 minutes to find and climb the hidden platform. A time of 20 seconds was allowed for them to stay on the hidden platform. The learning trial was performed daily for four days. The time to arrive the hidden platform during each session was considered as the escape latency time (ELT), an indicator of acquisition. The spatial probe test was performed without platform on the fifth day and the animal was given 120 seconds to explore the pool. The index of retrieval was a measure of the time consumed in the target quadrant (Q4) (10).

Novel object recognition (NOR) test

For this test, the designed equipment was a wooden, grey-colored box with two identical containers of equal size, each one made of glass and metal. The three consecutive phases of this test (habituation, training, and test) were carried out.

On the first day, the animals were placed in the open arena for habituation and allowed to explore it for 10 minutes. On day 2, in the training phase, the same procedure was repeated for 10 minutes for the exploration of box area with glass containers. On the next day, a novel environment was created by replacing one glass container with a similar metallic container (a novel object), and to each animal, 3 minutes time was given for exploration of the novel environment. The recording of the data was made for the time spent by each animal facing the novel object with touching and sniffing activities (11,12).

Neurobiochemical estimation

On the last day of all behavioural experiments (28th day), animals from each group were decapitated and the hippocampus area of brain was quickly dissected out. Using the High-performance liquid chromatography (HPLC) fluorescence detector, the levels of dopamine, 5-HT, and 5-HIAA in the hippocampus were measured (13). Prior to sample estimation, the HPLC system was calibrated using standards. The tissue homogenate was prepared in 0.1 mL hydrochloric acid and n-butanol, and cold centrifuged for 10 minutes at 2000 rpm. The supernatant was separated, and 0.08 mL of it was added to 0.2 mL of heptanes and 0.025 mL of 0.1M HCl in an Eppendorf tube. After shaking for 10 minutes, by centrifuging the mixture, the organic and aqueous phase were separated for the estimation of dopamine and 5-HT.

Estimation of dopamine

For this analysis, 0.005 mL HCl (0.04 M), 0.01 mL EDTA, and 0.01 mL iodine solution were added to 0.02 mL of the aqueous phase of the sample. To achieve the end point of the reaction, 0.01 mL of sodium metabisulfite solution in 5M NaOH and 0.01 mL of acetic acid (10M) were added just after 2 minutes. For 6 minutes, the above solution was heated to 100°C and then, cooled to ambient temperature. The recordings were made between 330 and 375 nm wavelength (13).

Estimation of serotonin (5-HT)

In brief, a stabilized serotonin sample was obtained by extracting the serotonin in ascorbic acid solution by the process of freezing and sonicating. Moreover, the ethanol enhanced the final fluorescence; to measure the fluorescence, a concentrated HCl medium was used (14).

Estimation of 5-HIAA

In order to estimate 5-HIAA, 0.023% octyl sodium sulfate and 0.0035% EDTA were added to the mobile phase of the sample. For achieving the final reaction, the process of heating the solution to 100°C for 6 minutes and cooling to ambient temperature was adopted. The readings for emission and excitation were recorded at wavelengths of 345 nm and 295 nm, respectively (15).

Statistical analysis

Data were subjected to one-way ANOVA with post hoc Tukey's multiple comparison test for analysis using GraphPad Prism 7.0. $P<0.05$ was considered as the minimum level of significance.

Results

Behavioral indices

Elevated plus maze test

This experiment revealed that, compared to normal rats, the rotenone-treated rats spent significantly more time in closed arms and performed a greater number of entries ($P<0.001$). The aforementioned parameters indicated a link between anxiety and PD brought on by rotenone. Furthermore, *S. wightii* at (400 mg/kg) alleviated the rotenone-induced anxiety-like behaviors ($P<0.001$) as compared to that of rotenone (Table 1).

Morris water maze test

In this experiment, the rats in the control group exhibited normal spatial learning ability and recognition memory. However, compared to the control animals, rotenone significantly prolonged the ELT ($P<0.001$), indicating impaired spatial learning function. The spatial probe test on the 5th day displayed reduced time spent in the target quadrant, indicating impaired memory ability. Notably, treatment with *S. wightii* exhibited a significant reversal of these effects of rotenone, indicating improved spatial learning and memory function ($P<0.001$) (Figure 2A).

Table 1. Effects of drugs on the time spent and the number of entries in various arms of the elevated plus maze

Groups	Time spent (s)		No. of Entries	
	Open arm	Closed arm	Open arm	Closed arm
I. Control	55.4 ± 8.75	136.25 ± 2.1	4.1 ± 0.55	2.78 ± 0.26
II. Rotenone (10 mg/kg)	16.92 ± 3.25$^{\#}$	230.29 ± 19.89$^{\#}$	1.84 ± 0.37$^{\#}$	4.92 ± 0.67$^{\#}$
III. *S. wightii* extract (400 mg/kg) + Rotenone (10 mg/kg)	51.98 ± 5.34***	115.52 ± 10.21***	4.31 ± 0.32***	4.67 ± 0.52***

The data represent the mean of six values. Applying one-way ANOVA with post hoc Tukey's multiple comparison tests for analysis; $^{\#}P < 0.001$ Comparison of vehicle vs. rotenone treated rats; $^{***}P < 0.001$ when compared with the rotenone group.

Novel object recognition test

This paradigm revealed that rotenone administration significantly decreased the time spent with the novel object to a highly significant extent ($P<0.001$) explaining impairment of recognition memory (Figure 2B). Furthermore, when compared to the rotenone-treated rats, animals with *S. wightii* treatment spent significantly more time ($P<0.001$) in the novel object recognition task, indicating enhanced recognition memory against rotenone-induced memory impairment.

Neurobiochemical analyses

In this experiment, in comparison to group I animals, with rotenone administration, there was a significant decrease in serotonin, dopamine, as well as 5-HIAA levels in the hippocampus ($P<0.001$). *S. wightii* treatment restored these rotenone-induced biochemical deficits significantly in contrast to rotenone treated animals ($P<0.001$). Again, these monoamine levels with *S. wightii* treatment were comparable to those of group I rats ($P>0.05$) (Figure 3).

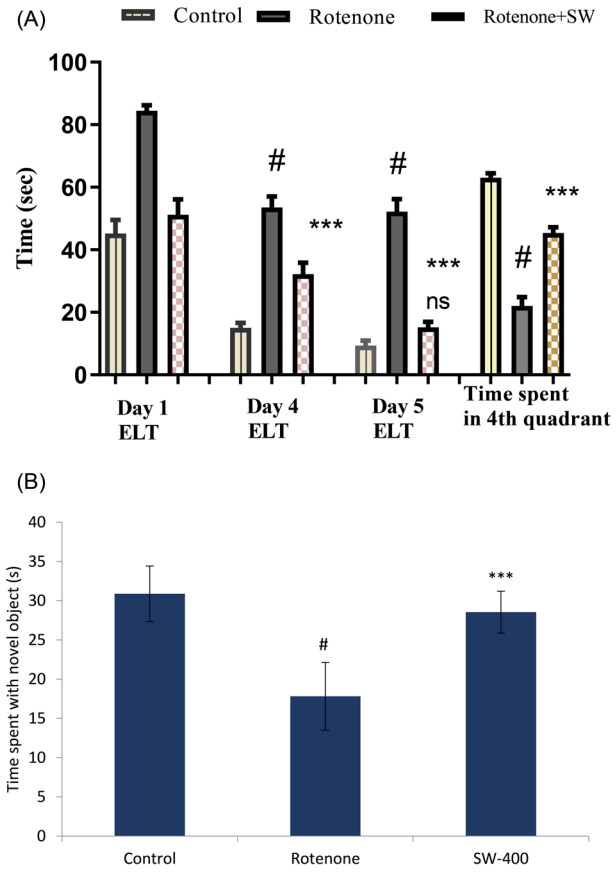

Figure 2. Effect of *Sargassum wightii* extract (400 mg/kg; SW-400) on rotenone-induced changes in (A) spatial working memory in the Morris water maze test and (B) the time spent with novel objects in the Novel Object Recognition test. Data are the mean of six values. Applying One-Way ANOVA with post hoc Tukey's multiple comparison test; #$P < 0.001$ as compared to the control rats. ***$P < 0.001$: test group vs. rotenone control group. ELT: escape latency time; ns: Not significant.

Discussion

The multifactorial pathogenesis of PD is a key challenge in its current therapeutic strategy. Additionally, recent evidence on the neuroprotective effect of bioactive compounds derived from plant sources is at the forefront of research exploring the benefits with minimal risk in the therapy of neurodegenerative diseases. The present research evaluated the protective effects of the methanol extract of *S. wightii* against non-motor symptoms developed in rotenone-induced parkinsonian rats. Rotenone, being highly lipophilic, easily crosses the blood-brain barrier, and its Parkinsonism-inducing effect in animals is well documented. Therefore, in the present investigation, we selected the rotenone-induced PD model in Wistar rats.

In our observation, *S. wightii* exhibited anti-anxiety effects in rotenone-induced PD, as evidenced by an increase in time spent and the number of entries into the open arms of the Plus maze. Our findings are in agreement with those of Venkateshgobi et al (16).

The Morris water maze test is a widely accepted model for screening spatial working memory, recognition memory, and performance (17). In this test, the animals receiving rotenone treatment exhibited a prolonged ELT to reach the target quadrant, indicating impaired spatial learning function. Further, in the spatial probe test, spending less time in the target quadrant indicated impaired memory ability, corroborating with that of earlier reports (18,19). It is noteworthy to observe that with *Sargassum* treatment, there was a reversal of these effects induced with rotenone, explaining the recovery of learning and memory. For the assessment of episodic memory, the novel object recognition test is an established

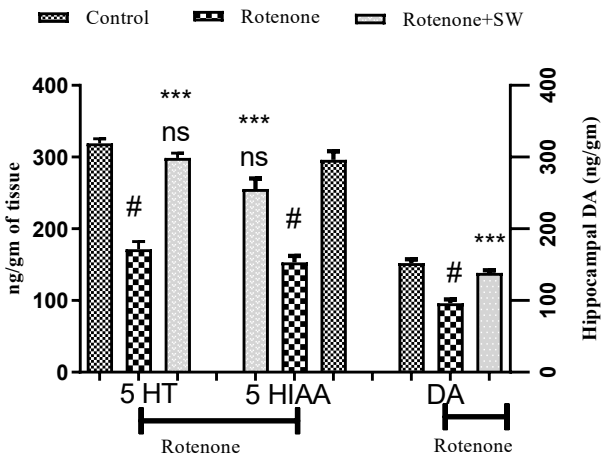

Figure 3. Effect of different drugs on 5-HT, 5-HIAA, and dopamine levels in hippocampal tissue. Values are mean ± SEM (n = 6). Applied one-way ANOVA with post hoc Tukey's multiple comparison test; #$P < 0.001$ comparison with the group I control rats; ***$P < 0.001$: test group vs. rotenone control group. ns (not significant): Test group vs. the control group. 5-HIAA, 5-hydroxyindoleacetic acid, 5-HT, 5-hydroxytryptamine; DA, dopamine.

model (20). In our observation, rotenone impaired recognition memory ability in rats while exploring novel objects, and *S. wightii* attenuated this recognition deficit as measured by the time spent exploring novel objects.

In neurobiochemical analyses, biochemical alterations, such as a decrease in hippocampal serotonin and dopamine levels observed in this experiment, could be correlated with anxiety-like behavior, and cognitive decline ascertained to rotenone. It is established that PD alters the neurotransmitter homeostasis in the striatal and extra-striatal regions of the brain (21). Evidence on the link between 5-HT and anxiety level supports our findings from the elevated plus maze test (22). A previous study report revealed a similar effect of rotenone supporting our findings (23). Interestingly, in our study, *S. wightii* (400 mg/kg) restored these neurotransmitter deficits induced with rotenone, indicating its anti-anxiety and memory enhancing effects, which are in line with other reports (12,23). More study reports revealing the neuroprotective effects of other seaweeds also support our hypothesis (24,25).

In our previous experiments, rotenone was found to decrease antioxidants (SOD, GSH) and increase lipid peroxides (MDA) in brain tissue homogenate. *S. wightii* amended the levels of biochemical alterations induced by rotenone. We have also observed the anti-inflammatory and strong antioxidant properties of the methanol extract of *S. wightii* in an in-vitro test. Moreover, we have reported the neuroprotective effect of the methanol extract of *S. wightii* against haloperidol-induced catalepsy (5). Therefore, we can explain that the antianxiety-like behavior and cognitive improvement shown by *S. wightii* could be by the virtue of its antioxidant and lipid peroxidation quenching activities. Thus, the oral administration of the methanol extract of *S. wightii* abrogated the anxiety-like behavior and impaired cognition induced with rotenone by replacing serotonin and dopamine in the hippocampus.

Conclusion

From this study, it can be concluded that *S. wightii* ameliorates non-motor symptoms of PD. This novel finding suggests further research in the line of investigating the role of specific neurochemicals and receptors involved in this neuroprotective effect.

Acknowledgment

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Authors' contribution

BR came up with the idea for the project and designed the experiment. SRP performed the experiment following all experimental guidelines and provided the data. BR and LMS supervised the work and reviewed the manuscript. IM and BM made the statistical interpretation and prepared the first draft of the manuscript. All authors had equal contributions in preparing the final version of the manuscript.

Conflicts of interests

Authors declare that there is no conflict of interest.

Ethical considerations

This experiment was conducted upon obtaining ethical clearance from the Institutional Animal Ethics Committee, MKCG Medical College, Berhampur, Odisha, India (Reg. No. 472/MKCG/CPCSEA). All the experimental procedures were performed strictly following the guidelines of CPCSEA formulated by the Ministry of Fishery, Animal Husbandry and Dairying, Government of India.

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