International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 12, Issue 3, 2020

Original Article

NEUROPROTECTIVE EFFECT OF METHANOLIC EXTRACT OF *SARGASSUM WIGHTII* ON HALOPERIDOL INDUCED CATALEPSY AND TARDIVE DYSKINESIA IN ALBINO RATS

S. ROUT[1], B. RATH[2], S. K. BHATTAMISRA[3], A. KUMAR[4], S. RATH[5]

[1,3]Pharmacology, Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, [*2] Pharmacology Fakir Mohan Medical College, Balasore, Odisha, [4]Pharmaceutical Chemistry, Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, [5]Anatomy, MKCG Medical College, Berhampur Odisha

Email: drbandana.rath@gmail.com

Received: 30 Nov 2019, Revised and Accepted: 06 Jan 2020

ABSTRACT

Objective: The present study was designed to evaluate the neuroprotective effect of methanolic extract of *Sargassum wightii* on haloperidol-induced catalepsy and tardive dyskinesia in Wistar albino rats.

Methods: In this study, thirty Wistar albino rats were randomly divided into six groups. Gr-I served as control. Haloperidol (1 mg/kg intraperitoneally) was administered to rats of Gr-II to Gr-V for twenty-one consecutive days to induce catalepsy and tardive dyskinesia. Animals of Gr-II to Gr-V were orally administered with vehicle, levodopa carbidopa combination (30 mg/kg), *Sargassum* extract 200 and 400 mg/kg respectively. All the drugs and vehicles were given orally one hour before haloperidol injection for twenty one consecutive days. The cataleptic scores were recorded using standard bar test. Tardive dyskinesia was assessed in terms of vacuous chewing movement (VCM) and tongue protrusion (TP) scores. After behavioural testing, all animals were sacrificed on twenty-second day and various biochemical parameters like MDA, SOD and GSH were estimated in brain tissue.

Results: Chronic administration of haloperidol significantly increased cataleptic scores, VCM and TP scores. ($p<0.001$) *Sargassum wightii* extract (400 mg/kg) significantly inhibited haloperidol-induced catalepsy, VCM and TP ($p<0.001$) Haloperidol increased MDA and decreased SOD and GSH in brain tissue to a highly significant extent ($p<0.001$) *Sargassum* extract at 400 mg/kg also significantly reversed the haloperidol-induced alteration in brain oxidative stress markers.

Conclusion: *Sargassum wightii* inhibits haloperidol-induced catalepsy and tardive dyskinesia. Thus it may be used as a unique therapeutic adjunct for the prevention of neuroleptic-induced extrapyramidal symptoms, however, it has to be explored more.

Keywords: *Sargassum wightii,* Catalepsy, Tardive dyskinesia, Oxidative stress, Albino rat

DOI: http://dx.doi.org/10.22159/ijpps.2020v12i3.36518. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps

INTRODUCTION

Neuroleptics used in the treatment of schizophrenia and other psychiatric illness are very often associated with extrapyramidal side effects like catatonia and tardive dyskinesia [1, 2]. Haloperidol, a typical antipsychotic produces extrapyramidal syndrome (EPS) like Parkinsonism, due to a blockade of D2 receptors within the striatum and reduced dopaminergic transmission [3, 4]. It produces a behavioural state of catalepsy and movement disorders like akathisia, dystonia, and at last, chronic tardive dyskinesia [5]. Hence haloperidol-induced catalepsy is considered as a robust model in rodents to evaluate nigrostriatal functions [6]. Evidence from many experimental and clinical studies also suggests that neuroleptics induce oxidative stress and cell death. Even lipid peroxidation byproducts in blood and cerebrospinal fluid are increased in patients with tardive dyskinesia [7, 8].

Marine algae or seaweeds have created a significant place in pharmaceutical and biomedical fields because they are recognized as the rich source of bioactive compounds and secondary metabolites like peptides, carotenoids, phenols, terpenoids, phlorotannins, flavonoids, fucoidans, phytosterols and glycolipids [9, 10]. Emerging researches have scientifically proved the anti-inflammatory, anticoagulant, antimicrobial, anticancer, antiviral, antioxidant, hypoglycaemic, antiulcer, neuroprotective and hepatoprotective effects of some of the seaweeds [11-14]. Several study reports have shown that the sulphated polysaccharide of some algae possesses free radical scavenging activity which might normalize lipid peroxidation [15, 16]. The chemical composition of *Sargassum* has been extensively studied however literature on its bioactivity is scarce. One earlier study report has shown that some of the constituents of *Hypnea musciformis* (one of the brown algae) raise the level of dopamine and its metabolites that alleviate the symptoms in Parkinsonism [17]. Hence it may be hypothesized that *Sargassum wightii*, another species of brown algae containing similar phytoconstituents could have a beneficial effect in Parkinsonism (PD). Interestingly, over the past decade, there is an amazing rise in researches on marine algae. But to our knowledge, scientific data on the neuroprotective activity of *Sargassum wightii* against PD are lacking. To bridge this knowledge gap, the current study was designed to evaluate the potential benefits of methanolic extract of brown seaweeds *Sargassum wightii* on haloperidol-induced catalepsy and tardive dyskinesia in wistar albino rats.

MATERIALS AND METHODS

Animal

Wistar albino rats of either sex (100-150g) were procured from Saha Enterprise, Kolkata and maintained in the animal house of Roland Institute of Pharmaceutical Sciences, Berhampur. Animals were housed and fed with standard pellet diet and water *ad libitum* and maintained under standard conditions temperature (24±1 °C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were kept under observation in the laboratory and allowed to acclimatize for one week before experimentation. With prior permission from the Institutional Animal Ethics Committee (IAEC), (Regd. No: 926/PO/Re/S/06/CPCSEA), Approval No-86/IAEC/RIPS/07.04.2017, handling and care of animals during experimentation were followed as per CPCSEA guidelines. The experiments were conducted between 10.00 to 16.00h.

Plant material

The plant material was a gift sample from Microbiotech limited, Gujrat India. The dried samples were coarsely ground to a fine

powder using electrical blender before extraction. To prepare the methanolic extract of *Sargassum,* 40 gm of powder sample was extracted with 400 ml of methanol by using soxhlet's apparatus for 72h. The extract obtained was dried in an evaporator and stored at 4 °C for further use [18]. Different concentration of drug solution was freshly prepared on the day of the experiment using 1% CMC suspension.

Chemicals

Haloperidol was purchased from Sigma, Aldrich USA; Levodopa and carbidopa combination: Glaxo Smithkline Pharmaceuticals, India; Carboxymethyl Cellulose(CMC), Thiobarbituric Acid (TBA), Trichloroacetic Acid (TCA), Dithiobis Nitro benzoic Acid (DTNB), phosphate buffer and all other chemicals used were of analytical grade and obtained from Himedia Laboratories Pvt Ltd, Mumbai, India.

Phytochemical screening of plant extract

The methanolic extract of *S wightii* was subjected to phytochemical screening test by the method described earlier [19] and it revealed the presence of polyphenols, terpenoids, tannins, flavonoids, polysaccharides like glycolipids, etc.

Acute toxicity test

Acute toxicity study was done as per OECD Guidelines 423 [Limit test]. The extract at doses 5, 50, 1000, 2000 mg/kg were given orally in stepwise form. The rats were observed for 24 h. There was no mortality or behavioral changes observed during the study period [20].

Experimental design

Haloperidol (1 mg/kg) was injected intraperitoneally for 21 d to induce catalepsy and tardive dyskinesia [21]. The test drugs and vehicles were given orally for 21 d, one hour before haloperidol injection. Thirty albino rats were randomly divided into five groups (n=6 in each group) to receive treatments as follows.

Group-I (control) received vehicle (1% CMC 0.5 ml/Rat).

Group-II (disease control) received 1% CMC.+Haloperidol

Group-III (standard) was treated with L-Dopa, carbidopa combination (30 mg/kg)+Haloperidol [21]

Group IV and V animals (test groups) received the methanolic extract of *Sargassum wightii* at the doses of 200 and 400 mg/kg respectively and were co-administered with Haloperidol (ip)

Then all the rats were subjected to behavioral and biochemical assessments. On the 22nd day, following behavioral assessment, the animals were sacrificed by cervical dislocation. The brains were removed, the forebrain was dissected and a 10% (w/v) of brain tissue homogenate was prepared in 0.1M phosphate buffer (PH-7.4)

Haloperidol-induced catalepsy

In this model, haloperidol 1mg/kg/ip was administered daily for 21 successive days to elicit a moderate to the high degree of catalepsy and it was scored as a measure of reduced ability to move and failure to maintain the correct posture using standard bar test. [22] Catalepsy was assessed in terms of time for which the animal maintained an imposed posture with both the front limb raised and resting on the 9 cm height wooden bar and scored accordingly. The endpoint of the catalepsy was considered when both the front paws were removed from the bar. If the animal maintained this position for 20 s or more it was said to cataleptic, given one point and every further 20 s was given more extra points (numbers). A cut-off time 300 sec was applied for scoring. The severity of catatonia was observed on the 7th, 14th and 21st days of haloperidol and test drug administration [23].

Haloperidol-induced tardive dyskinesia

For this experiment, 24 h after the last test dose (on the 22nd day), the animals were placed individually in small cages (30 x 20 x 30 cm). Then each animal was allowed to acclimatize to the observation cage for 10 min. The vacuous chewing movement (VCM) and tongue protrusion (TP) were observed for 5 min and scored. VCM was referred to as single mouth openings in the vertical plane not directed toward physical material. Individual tongue protrusions during oral dyskinesia were preceded by visible retraction of the tongue. If tongue protrusions or vacuous chewing movements occurred during a period of grooming, they were not taken into account [24].

Biochemical estimation

Lipid peroxidation assay (TBARS)

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the brain. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Okhawa *et al.* 1996. In which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colour complex having peak absorbance at 532 nm [25].

Reduced glutathione assay

Reduced glutathione (GSH) was determined by the method of Sedlak J *et al.* 1968. The procedure is based on the reduction of Ellman´s reagent by–SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically. (Shimadzu UV-1800) The amount of glutathione in tissue is expressed as µg/mg tissue [26].

Superoxide dismutase assay

The enzyme activity of superoxide dismutase in brain tissue was assessed using the method of Marklund and was expressed in terms of units of SOD activity/mg of tissue [27].

Statistical analysis

The observations were shown as mean±SEM. The data were analyzed using One-Way ANOVA followed by Tukey's post hoc test. Data analyses were performed using statistical software, Graph pad Prism 7. A probability value of less than 0.05 was considered as a minimum level of significance.

RESULTS

Phytochemical screening

The phytochemical screening of *Sargassum* extract revealed the presence of flavonoids, phenolic compounds, sterols, tannins, saponins, and glycolipids.

Acute toxicity test

On acute toxicity test, up to 2000 mg/kg body weight, no behavioral changes or mortality were observed within the study period.

Effect of methanolic extract of *S wightii* (SWE) on haloperidol-induced catalepsy

In this experiment, Haloperidol induced cataleptic score was significantly increased on 7th, 14th and 21st days of treatment in comparison to that of the control group ($p<0.001$). Standard drug L-dopa-carbidopa (30 mg/kg) decreased cataleptic score significantly on the 7th, 14th and 21st days of treatment as compared to that of haloperidol treated group on respective days. ($p<0.001$) However, pre-treatment with the test drug (SWE) at 200 mg/kg and 400 mg/kg reduced the cataleptic score to a highly significant extent only on the 14th and 21st days of observation when compared with the haloperidol control group on respective days. ($p<0.001$) table 1

Effect of administration *S wightii* on haloperidol-induced tardive dyskinesia in rats

On chronic administration, haloperidol increased the VCM and TP on the14th and 22nd days of treatment to a highly significant extent in comparison to that of the normal control group ($p<0.001$). With L dopa-carbidopa 30 mg/kg, the reference standard drug treatment, there was a highly significant decrease in the Haloperidol induced VCM and TP throughout the study period. ($p<0.001$) *Sargassum wightii* 200 mg/kg reduced the haloperidol-induced VCM and TP significantly only on the 22nd day of treatment as compared to that of

Haloperidol control. (p<0.01) However, pre-treatment of *S wightii* 400 mg/kg reduced VCM and TP to a highly significant extent both on the 14th and 22nd days of treatment when compared with the haloperidol treated control group (table 2).

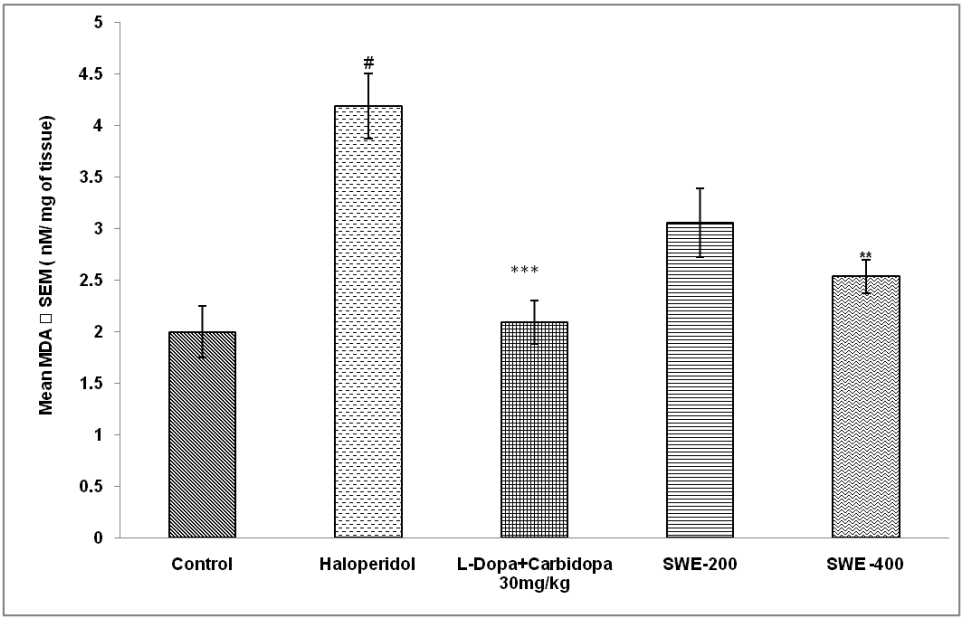

Fig. 1: Effect of *S wightii* on brain MDA levels. One way ANOVA followed by Tukey's multiple comparison tests was applied for analysis. #: p<0.001 when compared to the vehicle control group. **p<0.01; ***p<0.001; test group vs haloperidol control group

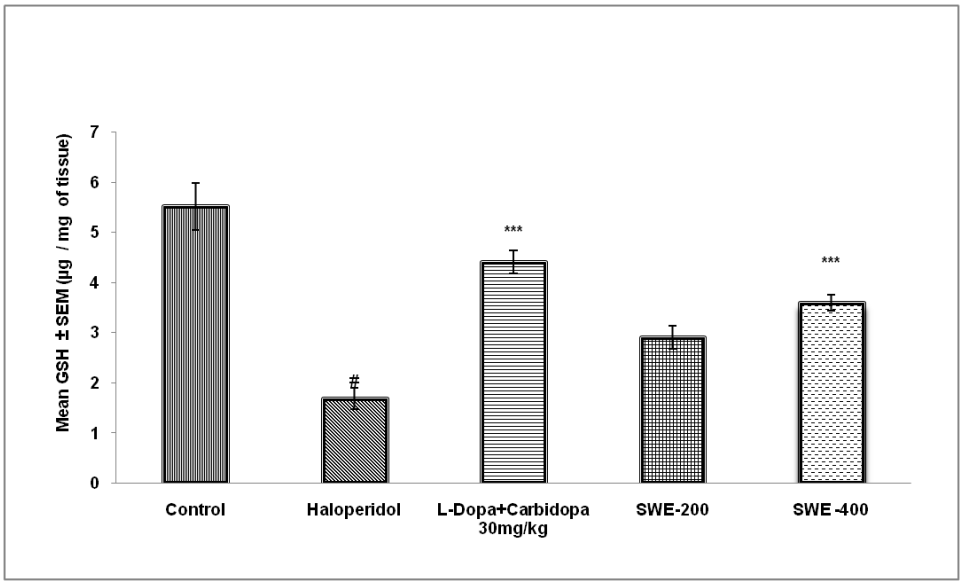

Fig. 2: Effect of methanolic extract of *Sargassum wightii* on GSH levels in the brain. One way ANOVA followed by Tukey's multiple comparison tests was applied for analysis. #: p<0.001 when compared to the vehicle control group. ***: p<0.001; test group vs haloperidol control group

Table 1: Effect of methanolic extract of *Sargassum wightii* on haloperidol-induced catalepsy

Group	Treatments	Mean cataleptic score		
		7th day	14th day	21st day
I	Vehicle	4.85±0.31	3.95±0.18	4.18±0.37
II	Haloperidol(1 mg/kg, ip)	117.4±2.38#	130.6±2.22#	126.7±5.57#
III	L Dopa-carbidopa (30 mg/kg)+Haloperidol	50.12±2.13***	36.03±1.21***	29.17±1.03***
IV	*S. wightii* (200 mg/kg)+Haloperidol	114.4±4.11	95.60±3.03***	73.90±3.03***
V	*S. wightii* (400 mg/kg)+Haloperidol	104.8±4.88	81.92±2.44***	55.98±2.65***

Values are expressed as mean±SEM, n=6. One way-ANOVA followed by Tukey's multiple comparison test was applied for analysis. #P<0.001 when compared with the vehicle group. ***: P<0.001 test group vs haloperidol control group.

Table 2: Effect of methanolic extract of *Sargassum wightii* on haloperidol-induced tardive dyskinesia

Group	Treatments	VCM/5 min		TP Frequency/5 min	
		14th day	22nd day	14th day	22nd day
I.	Vehicle	0.5±0.22	0.833±0.31	2.83±0.6	2.5±0.67
II.	Haloperidol(1 mg/kg ip)	14.33±1.50#	30.17±1.7#	26.83±3.02#	34.83±3.26#
III.	L-Dopa-carbidopa (30 mg/kg)+Haloperidol	4.67±0.61***	8.67±0.88***	8.5±1.8***	7.33±1.26***
IV	*S. wightii* (200 mg/kg)+Haloperidol	12.33±1.52	23.33±1.28**	18.83±1.96	24.17±2.41*
V	*S. wightii* (400 mg/kg)+Haloperidol	7.33±0.88**	17.83±0.83***	15.67±2.14**	18.33±3.18***

Values are expressed as mean±SEM, n=6. One way-ANOVA followed by Tukey's multiple comparison tests was applied for analysis. #P<0.001 when compared with the vehicle group. *: p<0.05 **: P<0.01; ***: P<0.001 test group vs haloperidol control group.

Effect of methanolic extract of *S wightii* (SWE) on haloperidol-induced changes in various biochemical parameters

On chronic haloperidol administration, the TBAR level in the brain was increased to a highly significant extent with a concomitant decrease in GSH and SOD in comparison to that of the control group (p<0.001). When compared with the haloperidol treated control group, L dopa-carbidopa (30 mg/kg-Gr III), as well as S wightii (400 mg/kg-GR V), significantly reduced the haloperidol-induced increase in TBAR with a concurrent significant rise in GSH and SOD. fig. 1, 2, 3.

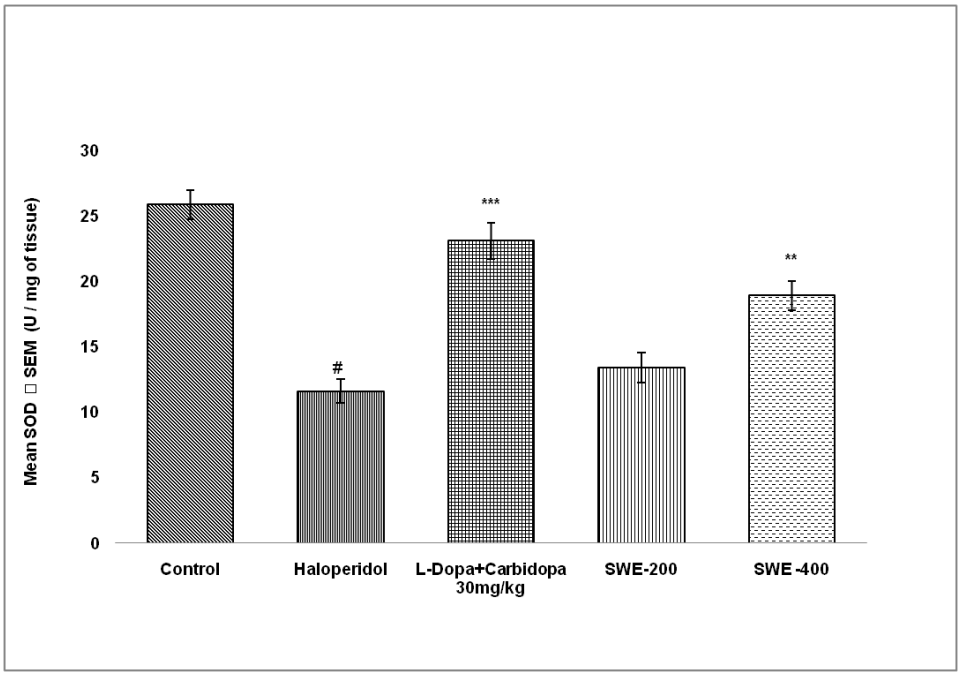

Fig. 3: Effect of *S. wightii* on SOD level in the brain. One way ANOVA followed by Tukey's multiple comparison tests was applied for analysis. #: p<0.001 when compared to the vehicle control group. ** p<0.01; ***: p<0.001; test group vs haloperidol control group

DISCUSSION

Haloperidol induced catalepsy (HIC) is a widely accepted animal model to test the effect of drugs modulating the extrapyramidal side effects of antipsychotic drugs [22, 28]. Haloperidol, a non-selective dopamine antagonist provides an experimental model in rodents simulating PD in human beings. It blocks dopamine receptors and interferes with dopamine transmission in the striatum. Thus the drugs which increase the dopamine transmission in the nigrostriatal pathway and inhibit HIC. Moreover, evidence in support of the "free radical hypothesis" proposes that the free radical byproducts of dopamine metabolism impart neurotoxic effects at basal ganglia and substantia nigra which explains one of the mechanisms involved in the process of neurodegeneration in neuroleptic-induced tardive dyskinesia and PD [29, 30].

In the present study, chronic administration of haloperidol (1 mg/kg ip) induced significant catalepsy. Treatment with methanolic extract of *S. wightii* (SWE) at both 200 mg/kg and 400 mg/kg doses, showed a protective effect against haloperidol-induced catalepsy significantly on 14thand 21stdays of the study period (table 1). It indicates that this seaweed extract can protect dopaminergic neurotransmission in the nigrostriatal pathway. Similar observations were made by B. S. Nishcal *et al.*, 2014 who have reported the protective effect of *Tribulus terrestris* plant against HIC and this effect was attributed to its dopamine facilitatory activity [31]. The anti-cataleptic activity of other plant extracts (*Ocimum sanctum, Ellagic acid*) also supports this hypothesis [32, 33] we also observed that the administration of *S wightii* (SWE) for 21 successive days reversed the haloperidol-induced tardive dyskinesia (TD) or orofacial dyskinesia. Even this effect of *S wightii* 400 mg/kg was comparable to that of L dopa and carbidopa (table 2). Our observations corroborate with that of Dhansekharan *et al.*, 2010 who have shown the amelioration of haloperidol-induced tardive dyskinesia by *Mucuna puriens* [24].

Oxidative stress generated as a result of mitochondrial dysfunction plays an important role in the pathogenesis of PD. Lipid peroxidation, a sensitive marker of oxidative stress impairs membrane function, distorts structural integrity and inactivates several membrane-bound enzymes present in all biological membranes. Superoxide dismutase (SOD) helps in neutralizing the free radical-induced toxic effects. Even depletion of glutathione is a positive correlate of the extent of neuronal loss. Evidences show that

a rise in MDA, decrease SOD and depletion of GSH in the brain result in neuronal loss (substantia nigra) and are important factors of the etiology of PD [34].

In the present study, haloperidol-treated animals exhibited an increase in the level of lipid peroxidation and decreased level of protective antioxidants such as GSH and SOD, suggesting a possible free radical generation. Previous studies have also reported that haloperidol-induced catalepsy is associated with oxidative stress [23, 24, 30] Treatment with methanolic extract of *S wightii* reversed the haloperidol-induced changes in oxidative stress markers like MDA, GSH and SOD significantly (fig. 1,2,3). Our observations are in accordance with that of Yuvraj *et al.*, 2014 who have shown the antioxidant activity of *S wightti* in an *in vitro* model [17]. Since the neuroleptic-induced extrapyramidal syndrome is correlated with an increase in free radical production it can be assumed that the antioxidant property of SWE could be another protective factor in the prevention of neurodegeneration in PD. Such correlation was also made by Perwez *et al.* who have shown the neuroprotective effect of *Mentha arvensis* against haloperidol-induced catalepsy in mice [23].

Studies on neuroprotective and antioxidant activities of *Sargassum* species have substantiated the role of various phytoconstituents. Presence of phenols, phytosterols, flavonoids, carotenoids, fucoxanthin, polysaccharides (fucoidan) and tannins in *Sargassum* (seaweeds) contribute to their promising antioxidant, anti-inflammatory and neuroprotective properties [35-37]. The plant of our research interest, *Sargassum wightii* contains the same phytoconstituents. Hence its neuroprotective effect observed in our research could also be attributed to its promising antioxidant activity. Further study is required in other experimental and clinical models of PD to elucidate the exact mechanism of neuroprotective activity of *S wightii*. In the present investigation, the isolation of individual phytochemicals and their effects on biogenic amines in the brain could not be done which directs future research.

CONCLUSION

Our study suggests that the *Sargassum wightii* inhibited the extrapyramidal features and reversed the altered oxidative stress parameters induced by haloperidol. Hence it can be a novel approach for the prevention of extrapyramidal side effects of neuroleptics. The naturally occurring phytochemicals present in *Sargasssum wightii* possessing neuroprotective and antioxidant properties with little toxicities pose a new insight for further research in this field.

ACKNOWLEDGMENT

The author is thankful to Roland Institute of Pharmaceutical Sciences and Department of Pharmacology, M K C G Medical College, Berhampur, Odisha for providing necessary facilities for this research work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

The corresponding author, Dr. B Rath designed the work and prepared the manuscript. Mrs. Sradhsini Rout performed the experiment and collected data, Dr. S K Bhattamisra supervised, Dr. A Kumar supported in the extraction and phytochemical analysis part of this work. Dr. S Rath performed the statistical analyses, reviewed the manuscript and edited.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

REFERENCES

1. Caroff SN, Hurford I, Lybrand J, Campbell EC. Movement disorders induced by antipsychotic drugs: implications of the CATIE schizophrenia trial. Neurol Clin 2011;29:127-48.
2. Carbon M, Kane JM, Leucht S, Correll CU. Tardive dyskinesia risk with first-and second-generation antipsychotics in comparative randomized controlled trials: a meta-analysis. World Psychiatry 2018;17:330-40.
3. Somani RS, Kasture VS, Kasture SB. Haloperidol inhibits (-) bicucullin induced seizures and bicucullin potentiates haloperidol-induced catalepsy in mice. Indian J Pharmacol 1993;31:434-6.
4. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. PET-analysis of central D-2 and D2-dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: relation to extrapyramidal side effects. Arch Gen Psychiatry 1992;49:538-44.
5. Marsden CD, Jenner P. The pathophysiology of extrapyramidal side-effects of neuroleptic drugs. Psychol Med Cambridge University Press 1980;10:55–72.
6. Kulkarni SK, Naidu PS. Isoniazid-induced orofacial dyskinesia in rats: an experimental model for tardive dyskinesia. Indian J Pharmacol 2001;33:286-8.
7. Lohr JB, Kuczenski R, Bracha HS, Moir M, Jeste DV. Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry 1990;28:535-9.
8. Patil R, Hiray YA, Kasture SB. Reversal of reserpine induced orofacial dyskinesia and catalepsy by *Nardostachys jatamansi*. Indian J Pharmacol 2012;44:340-4.
9. Liu L, Heinrich M, Myers S, Dworjanyn S. Towards a better understanding of medicinal uses of the brown seaweed Sargassum in traditional chinese medicine: a phytochemical and pharmacological review. J Ethnopharmacol 2012;142:591-619.
10. Yende SR, Harle UN, Chaugule BB. Therapeutic potential and health benefits of *Sargassum* species. Pharmacogn Rev 2014;8:1-7.
11. Fazeela Mahaboob Begum SM, Hemalatha S. Characterization, *in silico* and *in vitro* determination of the antidiabetic and anti-inflammatory potential of ethanolic extract of *Sargassum wightii*. Asian J Pharm Clin Res 2017;10:297-301.
12. Lee SG, Kang H. Neuroprotective effect of *Sargassum thunbergii* (Mertens ex Roth) kuntze in activated murine microglial cells. Trop J Pharm Res 2015;14:235–40.
13. Sumithra M, Arunachalam G, Chitra V, Gowri K. Neuroprotective effect of *Sargassum ilicifolium* turner C. Agardh on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rodents. Asian J Pharm Clin Res 2016;9:93-6.
14. Wen Ning Yang, Po-Wei Chen, Chun Yung Huang. Compositional characteristics and *in vitro* evaluations of antioxidant and neuroprotective properties of crude extracts of fucoidan prepared from compressional puffing-pretreated *Sargassum crasifolium*. Marine Drugs 2017;15:183.
15. Anwar E, Erianto H, Putri HSS. Preparation of powder from brown seaweed (*Sargassum plagyophyllum*) by freeze-drying with maltodextrin as a stabilizer. Int J Appl Pharm 2018;10:348-53.
16. Praveen NK, Chakraborty K. Antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from brown marine macroalgae padina sp. From the gulf of mannar of peninsular India. J Coastal Life Med 2013;1:38-48.
17. Najam R, Ahmed SP, Azhar I. Pharmacological activities of *Hypnea musciformis*. Afr J Biomed Res 2010;13:69–74.
18. Yuvaraj Neelakandan, Venkatesan A. *In vitro* anti-tumor, anti-inflammatory, antioxidant and antibacterial activities of marine brown alga *Sargassum wightii* collected from the gulf of mannar. Global J Pharmacol 2014;8:566-77.
19. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Pune: Nirali Prakashan. 47th ed; 2011. p. 6.15-6.19.
20. OECD Guidelines for testing of chemicals 423; 2001.
21. Nade VS, Kawale LA, Zambre SS, Kapure AB. Neuroprotective potential of *Beta vulgaris* L. in Parkinson's disease. Indian J Pharmacol 2015;47:403-8.
22. Ferre S, Guix T, Prat G, Jane F, Casas M. Is experimental catalepsy properly measured? Pharm Biochem Behav 1990;35:753-7.
23. Parwez Ahmad MD, Arshad H, Kalam NA, Anshu M, Hasin MD, Shadma W. Effect of the aqueous extract of *Mentha arvensis* on haloperidol-induced catalepsy in albino mice. J Clin Diagnostic Res 2012;6:542-6.

24. Sivaraman D, Ratheesh KS, Palayan M. Effect of ethanolic seed extract of *Mucuna pruriens* on haloperidol-induced tardive dyskinesia in rats. Int J Pharm Sci Rev Res 2010;3:106-13.
25. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by the thiobarbituric acid reaction. Anal Biochem 1979;95:351-4.
26. Sedlak J, Lindsay RH. Estimation of total protein (bound) and the nonprotein sulfhydryl groups in tissues by using Ellman's reagent. Anal Biochem 1968;25:192-205.
27. Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur J Biochem 1974;47:469-74.
28. Elliott PJ, Close SP, Walsh DM, Hayes AG, Marriott AS. neuroleptic-induced catalepsy as a model of Parkinson's disease. I. Effect of dopaminergic agents. J Neural Transm Park Dis Dement Sect 1990;2:79-89.
29. Aurel Popa Wagner, Smaranda Mitran, Senthilkumar Sivanesan, Edwin Chang, Ana-Maria Buga. ROS and brain diseases: the good, the bad, and the ugly. Oxidative Medicine and Cellular Longevity; 2013. p. 1-14.
30. Rajaram C, Reddy KR, Chandra Sekhar KB. Neuroprotective activity of *Tephrosia purpurea* against haloperidol-induced Parkinson's disease model. Pharmacologia 2015;6:125-30.
31. Nishchal BS, Rai S, Prabhu MN, Ullal SD, Rajeswari S, Gopalakrishna HN. Effect of *Tribulus terrestris* on haloperidol-induced catalepsy in mice. Indian J Pharm Sci 2014;76:564-7.
32. S Pemminati, V Nair, P Dorababu, HN Gopalakrishna, MRSM Pai. Effect of ethanolic leaf extract of *Ocimum sanctum* on haloperidol-induced catalepsy in albino mice. Indian J Pharmacol 2007;39:87-9.
33. Dinesh Dhingra, Nidhi Gahalain. Amelioration of haloperidol-induced orofacial dyskinesia and catalepsy by *Ellagic acid* in rats. Int J Res Ayurveda Pharm 2016;7(Suppl 2):222-7.
34. Jitendra O Bhangale, Sanjeev R Acharya. The anti-parkinson activity of petroleum ether extract of *Ficus religiosa* (L.) leaves. Adv Pharmacol Sci 2016;1-9. http://dx.doi.org/10.1155/2016/9436106.
35. Susete Pinteus, Marco FL Lemos, Joana Silva, Celso Alves, Agnieszka Neugebauer, Rafaela Freitas, *et al*. An insight into *Sargassum muticum* cytoprotective mechanisms against oxidative stress on a human cell *in vitro* model. Marine Drugs 2017;15:353.
36. Bogie J, Hoeks C, Schepers M, Tiane A, Cuypers A, Leijten F, *et al.* Dietary *Sargassum fusiforme* improves memory and reduces amyloid plaque load in an Alzheimer's disease mouse model. Nature News. Nature Publishing Group; 2019.
37. Schepers M, Martens N, Tiane A, Vanbrabant K, Liu HB, Lütjohann D, *et al*. Edible seaweed-derived constituents: an undisclosed source of neuroprotective compounds. Neural Regen Res 2019;15:790-5.