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Original Article

Evaluation of Anti-Depressant Activity of *spirulina, a blue-green algae* on Chronic Unpredictable Stress Induced Depression in Rats

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ABSTRACT

Background: Depression is a crippling and pervasive illness that can affect a person in many different ways. It can be identified by certain symptoms, such as modifications in behaviour, psychological functioning, and brain physiology. **Materials and Methods:** One of the animal models for depression has been the Chronic Unpredictable Mild Stress (CUMS) paradigm. In this study, spirulina algae, which contains tryptophan, was chosen and tested for its anti-depressant effectiveness in the CUS model using Fluoxetine as the gold standard. Wistar rats were chosen to undergo the CUS process for 28 days, and throughout that time, the test drug was given at doses of 400 mg/kg. **Results:** Behavioural and biochemical parameters were analysed, and has shown significant changes. **Conclusion:** When compared to the CUS group, spirulina algae at the tested doses had a substantial impact on behavioural and metabolic testing. These findings demonstrated that Spirulina algae specifically exhibited an *in vivo* anti-depressant-like effect.

Keywords: Antidepressant, Spirulina algae, Force swim test, Tail suspensstion test, Sucrose preference test, Chronic Unpredictable Stress.

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INTRODUCTION

With a lifetime frequency of 17% worldwide, major depression, also known as Major Depressive Disorder (MDD), is a neuropsychiatric illness that is frequently diagnosed.[1-3] By 2020, serious depression is expected to overtake cardiovascular illnesses as the second leading cause of disability worldwide. The majority of the drugs that change the activity of monoaminergic neurotransmitter systems have been the focus of established treatment regimens for depression and other associated diseases. One of the widely accepted hypotheses is functional deficit of norepinephrine and serotonin in the brain which leads to depressive state.[4-6]

It has become clear that depression affects about 350 million individuals worldwide and is a widespread disorder. According to the WHO's forecast, the prevalence of depression is anticipated to increase by 113% yearly and rank as the second most common condition worldwide by 2020.[7,8] Young adulthood is said to be a censorious time when depression is more likely to occur due to tasks, family issues, transitions, and changes in social roles. Continuous stress beginning in adolescence has been linked to anomalies in the endocrine system and brain function as well as long-term somatic consequences in adulthood, according to studies.[9-11] The main region of the brain involved in controlling emotion, regulating in learning, and enhancing memory is the hippocampus, which is the primary region responsible for these functions. Additionally, the focalization of depression is complicated and primarily involves CNS and neuroendocrine dysfunction, in addition to neurobiological and morphological alterations across the centres of the brain that are noticeably vulnerable to stress.[12,13]

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Multicellular and filamentous blue-green algae known as Arthrospira (Spirulina) are becoming more and more popular in the health food sector and as a protein and vitamin addition to aquaculture diets.[14,15] It has a very high macro- and micronutrient content, can be picked and processed with ease, and grows in water. It is still harvested from natural water, dried, and used as a primary source of protein in many African nations. In many Asian countries, it is used as a protein supplement and a health food, and it has become quite popular in the market for human health foods.[16-18]

Chemical analysis of spirulina has demonstrated that it is a superb source of proteins, vitamins, lipids, minerals, carbs, nucleic acids, enzymes, and colours. Human nutrition can benefit from it.[19] Leucine (540 mg per 10 m), valine (400 mg per 10g), isoleucine (350 mg per 10g), and tryptophan (90 mg per 10g) have the highest concentrations of essential amino acids in spirulina, which contributes to its biological value of 20 and the nutritional value of its proteins.[20]

As *Spirulina* has various chemical constituents which has been reported to improve the physiological condition a human. We want to assess the antidepressant efficacy of spirulina on chronic unpredictable stress induced depression in rats in this study.

MATERIALS AND METHODS

Acute toxicity study

Following the recommendations of the OECD (Organisation for Economic Co-operation and Development), the study-up and procedure for acute toxicity were completed. If an animal is given a certain dose and dies, the next animal is given a lower dose, and if the animal survives, the next animal is given a larger dose for the remaining animals.[21] Spirulina suspension at the top limit dose of 400 mg/kg was given orally to rats. After the dose, each animal was watched closely. Mortality and clinical signs were seen. such include modifications to the eyes, mucous membranes, and skin.

Procurement of Animals

Prior to the trial, male Wistar rats weighing 200–220 g were housed in polypropylene cages for two weeks after being obtained from the Bangalore Biogen Laboratory Enterprises Animal Facility. Animals received a regular pellet diet and unrestricted access to water the week before the tests. Additionally, they were kept in a 12 hr light/dark cycle with constant temperatures of 22–20°C and relative humidity values of 50–10%. All trials in this case were held from 10:00 AM to 5:00 PM. The Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy, Tiruchanoor and Tirupathi approved all of the procedures and practises detailed in the current paper. The Indian government obtained approval for the use of animal experimentation methods from the CPCSEA (SPSP: 1016/PO/Re/S/06/CPCSEA/2020/002) Committee for the Purpose of Control and Supervision of Experiments on Animals.

Procurement of test compounds

Fluoxetine was obtained from Glenmark Pharmaceuticals, India. Spirulina, SOD standard, Glutathione reduced, DTNB, Epinephrine bitartarate and Tris buffer were procured from Sigma-Aldrich, Bangalore, India. Disodium EDTA, Potassium Dihydrogen Phosphate, Sodium Chloride, sucrose, Sodium bicarbonate, Sodium carbonate, Hydrogen peroxide, Trichloroacetic acid, Sodium phosphate, formaldehyde, Normal saline and hydrochloric acid were obtained from different agencies.

Drug treatment

An effective positive control for an antidepressant's effectiveness is the selective serotonin reuptake inhibitor fluoxetine. The four groups of six animals were: vehicle control, CUMS plus vehicle, CUMS plus spirulina (400 mg/kg), and CUMS plus fluoxetine (10 mg/kg). in each. Fluoxetine and spirulina are taken orally once daily for four weeks, between 10:00 and 10:30 a.m.

Test Drug Materials

The spray dried powder of *Spirulina Platensis* is employed in this experiment was obtained from Pondicherry Spirulina farms, a South-India; Natural Food Company specialized in the cultivation, manufacturing and marketing of spirulina.

Chronic Unpredictable Stress model

There were four groups of rats. (1) The control group; (2) the disease prevention group; (3) the standard group; and (4) the drug test group with fluoxetine (10 mg/kg) serving as the positive control, CUS was treated as usual. Six animals make up each group (n=6), and each group is housed in a separate cage.[22] The rats in the CUS group were exposed to various stressors, while the rats in the control group were kept alone in their cages for 28 days (four weeks).The stressors include bright light (30 min), food deprivation (30 min), water deprivation (30 min), inverse dark light cycle (30 min), acoustic stimulation (120 decibels), cold swimming (4°C, 5 min), hot environment (40°C), crowding (30 min), tail pinch (1 min, 1 cm from the end of the tail), foot shock (25 V, 30 min), and forced swimming (30 min). Rats were exposed to these stimuli throughout the day at various periods to avoid predictability. After 24 hr had passed since the final foot shock on day 29, behavioural testing was initiated (Che Y and Zhou Z C *et al.*, 2015).

Instruments

Ever Shine's homogenizer (model number 607), Remi's cooling centrifuge (model number C-24 BL), Inverse Dark Cycle LED light (80 Lux), Bright light (10,000 Lux), Acoustic stimulation

siren, and pole-climbing are some examples of the equipment used in this experiment.

Experimental protocol

To evaluate the anti-depressant activity, the experimental animals were split into four groups, each with six animals (n=6). After the 28-day treatment period was over, group IV received fluoxitine (10 mg/kg, p.o.) to help with anti-depression. The treatment and rat groups are depicted in Tables 1 and 2.

Behavioral parameters

Sucrose preference test

At the conclusion of four weeks, the test is conducted. In all trials, 1% sucrose solution is exposed for two days prior to the initial test of sucrose preference to allow subjects to become acclimated to it. After the initial test, the only liquid that could be consumed was sucrose solution for 48 hr; following that, two days of limitless access to food and water were permitted. The rats were dehydrated for 16 hr prior to the baseline test on day 0 of sucrose preference [Figure 1]. The experiment was carried out in the rat's cage at home using two bottles, one containing tap water and the other 1% sucrose solution. The volume difference after an hour is regarded as rat intake.[23]

Using the following formula, the total intake was determined as the sum of the intake of water and sucrose solution, and the preference for sucrose was expressed as the ratio of sucrose intake to total intake:

$$\text{Sucrose preference} = \frac{\text{sucrose consumption}}{\text{Water consumption} + \text{sucrose consumption}} \times 100\%$$

Sucrose preference test

Forced swim test

The most popular behavioural model for screening antidepressant-like behaviour in rats is the forced swim test. It was first put forth in 1998 by (Porsolt RD *et al.*). Rats were made to move independently in a swim test device (25, 15, 25 cm) that contained potable water that was kept at a temperature of 260°C and rose to a height of 15 cm [Figure 2]. Since used water has been shown to alter rat behaviour, it is necessary to change it after each animal has been forced to swim. Throughout the first 5 min of the trial, every animal makes amazing motions. The final 4 min of a 6 min test session were used to calculate the total amount of immobility in seconds. When rats only moved to maintain their heads above water and made no other attempts to escape from the water, they were considered immobile. It was discovered that the rats were immovable when they gave up and remained motionless in the water, only moving enough to keep their heads above the surface.[24]

Tail suspension test

The tail suspension test is a quick and easy way to evaluate potential antidepressants, claims. It has been suggested that the immobility displayed by mice under unavoidable stress is a reflection of behavioural despondency, It may be comparable to human depression illnesses. Clinically effective antidepressants decrease the immobility that rats exhibit while dangling by their tails after making frantic and unsuccessful attempts to escape [Figure 3].

Doses are given once day for a week. On the seventh day, an hour after the test and standard medication had been given, rats were dangled on the edge of a table 50 cm off the ground. A centimetre or so away from the tail's tip was where the adhesive tape was placed. The duration of immobility was calculated over a 6 min period (Zomkowski AD *et al.*, 2002).

An animal was deemed immobile if its body showed no signs of movement and it hung passively. Parameters in Brain tissue homogenate Tissue processing.

Estimation of *in vivo* antioxidant parameters

Preparation of homogenate

Animals were beheaded and their brains were removed at the conclusion of the experiment. The brain was weighed, and the homogenate was made in the manner described below. 1. In ice-old 10 mm tris HCL buffer (to pH 7.4) at a concentration of 10% (w/v), the excised brain tissue was minced and homogenised for 25 strokes with a tight teflon pestle of glass homogenizer.

Table 1: Treatment schedule for assessing the chronic unpredictable stress.

Group	Name	Stress induced	Purpose
I	Vehicle control (n=6)	0.3% (Carboxy methyl cellulose).	Serves as normal.
II	Disease control (n=6)	Chronic unpredictable stress induced for 28 days.	Disease control.
III	Standard- drug (n=6)	Chronic unpredictable stress induction for 28 days + Standard drug (Fluoxitine 10 mg/kg/day).	Serves to effect of test drug.
IV	Test-drug (n=6)	Chronic unpredictable stress induced for 28 days + Test drug (dose 400 mg/kg/day).	Serves to evaluate of Test drug.

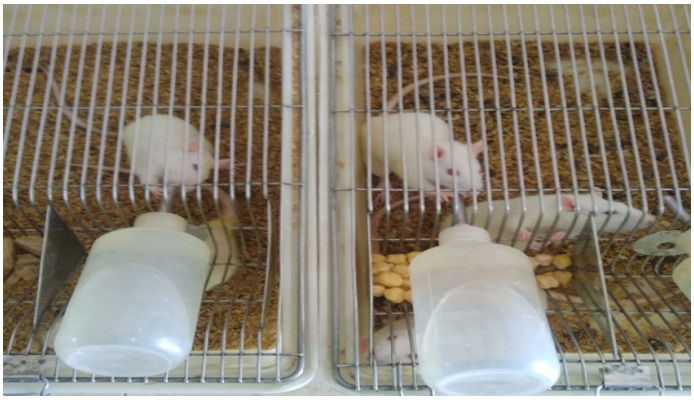

Figure 1: Sucrose preference test.

Figure 2: Forced swim test.

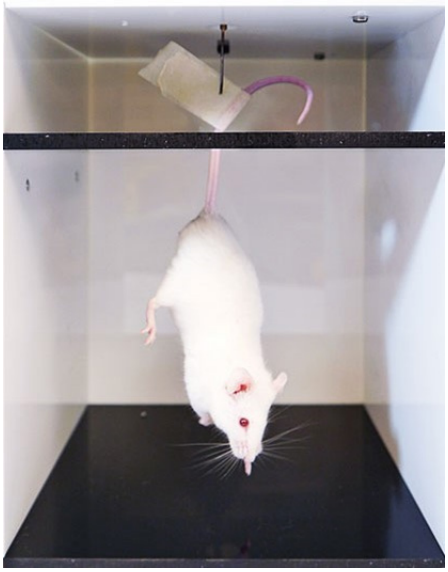

Figure 3: Tail suspension test.

After being cut into thin slices, the tissue was chilled in the 0.25M cold sucrose solution. After centrifuging at 5000 rpm at 200°C temperature, the clear supernatant was then separated. This was used to determine the levels of Super Oxide Dismutase (SOD), reduced Glutathione (GSH), Catalase (CAT), and Lipid Peroxidation (LPO). The lengthy homogenization procedure was done under hypnosis with the intention of severely harming the cells' ventricular surfaces.

In vivo antioxidant parameters

Measurement of superoxide dismutase SOD was estimated by (ARRON GP *et al.* 1972). The rate of epinephrine autooxidation and its vulnerability to SOD inhibition increased as pH rose from 7.8 to 10.2. Due to the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O_2 supplied, xanthine oxidase produces O_2. There are at least two distal pathways by which epinephrine could experience autooxidation, although only one of them involves oxygen and is hence inhospitable since it results in a free radical chain reaction.

Reagents

Carbonate buffer (0.05 M, pH 10.2): The final volume was created by adding distilled water after dissolving 16.8 g and 22 g of sodium bicarbonate in 500 mL of water.

Ethylene Diamine Tetra Acetic Acid (EDTA) (0.49 M): 1.82 g of EDTA was dissolved in 1000 mL of distilled water.

Epinephrine (3 mM): 9.9 g of epinephrine bitartarate was dissolved in 10 mL of 1M HCl solution.

Procedure

All the chemicals were kept at a cool temperature as 0.5 mL of the sample was diluted with 0.5 mL of distilled water, 0.25 mL of ethanol, and 0.5 mL of chloroform. Before centrifuging the mixture for 20 min at 2000 rpm, the mixture was mixed for 1 min. The supernatant's enzyme activity was measured. Additionally, 0.5 mL of EDTA (0.49 M) and carbonate buffer (0.05 mL M, pH 10.2) were added. Using 0.4 mL of epinephrine to start the reaction, a change in optical density/min was noted at 480 nm. In terms of units/mg protein change in optical density/min, SOD activity was determined. In order to limit the transfer of epinephrine to adrenochrome by 50%, the enzyme unit is supplied. 10-125 units of SOD were used to create the calibration curve [Table 3].

Calculation

$$SOD = \frac{0.025 - Y}{Y \times 50} X\ 100$$

Where Y= Final reading – Initial reading

Measurement of reduced GSH

The method developed by Ellman M. in 1959 was used to test glutathione.

Principle

Glutathione's sulphydryl groups combine with DTNB [5, 5-dithiobis-(2-nitrobenzoic acid)] to generate a coloured complex that was detected by a colorimeter at 412 nm.

Reagents

Trichloro Acetic Acid 10% (TCA): 10 gm of TCA was dissolved in 100 mL of distilled water.

DTNB: 12 mg of DTNB was dissolved in 20 mL of distilled water.

Phosphate buffer (pH 7.4).

Procedure

Homogenate (w/v) and 10% TCA were mixed together and centrifuged to separate the proteins. To 0.01 mL of this supernatant, 2 mL of phosphate buffer (pH 7.4), 0.5 mL DTNB, and 0.4 mL of double-distilled water were added. After 15 min of vortexing the liquid, the absorbance was measured at 412 nm. Ellman *et al.* (1959) stated that GSH values were expressed as moles GSH mg protein [Table 4].

Table 2: Protocol for Chronic Unpredictable Stress (CUS). Daily plans to combat unpredictable, chronic stress. utilized on the day of treatment stressor.

Test	Blank
Day 1	Bright light (30 min)
Day 2	Food deprivation (24 hr)
Day 3	Acoustic stimulation (120 db, 30 min)
Day 4	Inverse light dark cycle
Day 5	Inverse light dark cycle
Day 6	Foot shock (25 V, 30 time)
Day 7	Hot environment (40°C) and overcrowding (30 min)
Day 8	Foot shock (25 V, 30 time)
Day 9	Tail pinch (1 min, 1 cm from the end of the tail)
Day 10	Bright light (30 min)
Day 11	Water deprivation (24 hr)
Day 12	Food deprivation (24 hr)
Day 13	Cold swimming (4°C, 5 min)
Day 14	Acoustic stimulation (120 db, 30 min)
Day 15	Foot shock (25 V, 30 time)
Day 16	Hot environment (40°C) and overcrowding (30 min)
Day 17	Tail pinch (1 min, 1 cm from the end of the tail)
Day 18	Inverse light dark cycle
Day 19	Food deprivation (24 hr)
Day 20	Bright light (30 min)
Day 21	Cold swimming (4°C, 5 min)
Day 22	Cold swimming (4°C, 5 min)
Day 23	Acoustic stimulation (120 db, 30 min)
Day 24	Water deprivation (24 hr)
Day 25	Tail pinch (1 min, 1 cm from the end of the tail)
Day 26	Bright light (30 min)
Day 27	Hot environment (40°C) and overcrowding (30 min)
Day 28	Foot shock (25 V, 30 time)

Calculation

$$\text{Reduced glutathione} = \frac{y - 0.0046}{0.0034}$$

Where, Y = Final reading – Initial reading,

Measurement of catalase

Catalase activity was measured by the method (Claiborn CD *et al.*, 1979).

Principle

The peroxidase activity of catalase allows it to react with H_2O_2 and H donors (methanol, ethanol, formic acid, or phenols) to produce water and molecular oxygen with high efficiency.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O_2 + O_2$$

Table 3: Test outcomes and ineffective remedies.

Test	Blank
0.5 mL of diluted homogenate	0.5 mL of distilled water
0.5 mL of distilled water	-
0.25 mL of ethanol	0.38 mL of ethanol
0.5 mL of chloroform	0.15 mL of chloroform
Shake for 1 min, centrifuge at 2000 rpm, and separate the supernatant	
0.5 mL of supernatant	1.5 mL of above mixture
1.5 mL of carbonate buffer	1.5 mL of carbonate buffer
0.5 mL of EDTA	1.5 mL of EDTA

Table 4: Test and blank solutions.

Blank	Test
Take 1 mL of distilled water	Take 1 mL of brain homogenate
Add 1 mL of 10% TCA	Add 1 mL of 10% TCA and subject the mixture for cooling for 10 min followed by centrifuging at 2000 rpm.
Take 0.01 mL of above mixture	Take 0.01 mL of supernatant
Add 0.5 mL of DTNB	Add 4 mL of DTNB
Add 2 mL of phosphate buffer of PH 7.4	Add 1.5 mL of phosphate buffer of pH 8.

Reagents

Phosphate buffer (50 mM/L; pH 7.0).

Make up to 1000 mL of KH2PO4 solution by dissolving 6.81 g in water. Make up to 1000 mL of NaH_2PO_4 $2H_2O_2$ by dissolving 8.9 g in water.

In a ratio of 1:1.5, combine solutions (a) and (b).

Hydrogen peroxide (30 mM/L).

Dilute 0.34 mL of 30% hydrogen peroxide with phosphate buffer up to 100 mL.

Procedure

A cuvette with 1.9 mL of 50 mM phosphate buffer (pH 7) and 0.1 mL of supernatant total was used. 30 mM, newly produced H_2O_2 was added in a volume of 1 mL to begin the reaction. At 240 nm, the rate of H_2O_2 oxidation was quantified spectrophotometrically. Claiborne *et al.* (1985) reported catalase values as micromoles of H_2O_2 consumed/min/mg protein.

Calculation

$$\text{Catalase} = \log \frac{A}{B} \times 2297.3$$

Where, A= Initial absorbance,

B= Final absorbance,

Units= μ moles of H_2O_2consumed/min/mg.

Table 5: Test and blank solutions.

Test	Blank
10% tissue homogenate	1 mL of distilled water
0.5 mL of 30% TCA	0.5 mL of 30% TCA
0.5 mL of 0.8% TBA	0.5 mL of 0.8% TBA
Water bath for 30 min at 80°C	
Ice cold water for 30 min	
Centrifuged at 3000 rpm for 15 min	

Evaluation of pro-oxidant

Measurement of lipid peroxidation (Malondialdehyde formation).

Ohkawa H *et al.* (1979) described how to quantify TBARS, a lipid peroxidation indicator.

Principle

One of the secondary byproducts of lipid peroxidation to interact with Thiobarbituric Acid (TBA) is malondialdehyde. The basic idea behind this procedure is that MDA reacts with thiobarbituric acid at a higher temperature and in an acidic environment to produce a pink MDA-(TBA) complex that can be measured spectrophotometrically at 532 nm.

Reagents

Thiobarbituric acid (0.8%).

8 g of Thiobarbituric acid was dissolved in 100 mL of 1M tris hydrochloride, pH7.

Trichloroacetic acid (30%).

30 g of TCA was dissolved in 100 mL of distilled water.

Procedure

1 mL of suspension medium was created using the 10% tissue homogenate. After that, it received 0.5 mL of 0.8% TBA reagent and 0.5 mL of 30% TCA. The tubes were covered with aluminium foil and submerged in a water bath that was shaking at 80°C for 30 min. After 30 min, the tubes were taken out and kept in ice-cold water for an additional 30 min. These were centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was measured at room temperature with a suitable blank [Table 5]. The blank consists of 1 mL of distilled water, 0.5 mL of 30% TCA, and (MDA)/mg protein (Ohkawa *et al.*, 1979).

Calculation

$$\text{Lipid peroxidation} = \frac{y+0.002}{0.0026086}$$

Where, Y= Absorbance differences of final (after 3 min) and initial reading of test sample.

Table 6: Effect of spirulina on duration of immobility taken to forced swim test.

Sl. No.	Groups	Duration of immobility time (mints)				
		0th day	7th day	14th day	21st day	28th day
1	Normal	2.28±0.02	2.27±0.04	2.27±0.05	2.28±0.02	2.29±0.01
2	Disease control	2.27±0.03	2.55±0.02***	3.10±0.07***	3.32±0.01***	3.56±0.02***
3	Standard	2.29±0.02	2.40±0.01##	2.37±0.01###	2.34±0.01###	2.31±0.01###
4	Test	2.26±0.02	2.43±0.01#	2.41±0.02###	2.35±0.01###	2.33±0.01###

All values are expressed in mean ± SEM n=6.*** indicates p<0.001 when disease control group compared with normal group.### indicates p<0.001, ## indicates p<0.01, # indicates p<0.05 when standard and test groups are compared with disease control group.

Table 7: **Effect of spirulina on immobility time tail suspension test.**

Sl. No.	Groups	Immobility time (seconds)				
		0th day	7th day	14th day	21st day	28th day
1	Normal	85.0±5.4	87.50±4.6	83.3±6.5	85.0±6.1	83.3±4.94
2	Disease control	84.1±5.0	148.3±5.1***	153.3±4.2***	160.0±2.8***	167.5±4.2***
3	Standard	87.1±1.7	113.0±2.3###	108.2±2.12###	103.8±1.6###	95.8±1.30###
4	Test	86.1±3.2	124.5±1.4##	121.3±2.14###	114.7±2.0###	108.2±1.4###

All values are expressed in mean ± SEM n=6.*** indicates p<0.001 when disease control group compared with normal group.### indicates p<0.001, ##indicates p<0.01 when standard and test groups are compared with disease control group.

Table 8: **Effect of spirulina on sucrose preference test.**

Sl. No.	Groups	Sucrose preference (%)	
		0th day	28th day
1	Normal	84.1±3.27	82.50±3.09
2	Disease control	83.3±3.3	41.6±2.47***
3	Standard	83.3±4.94	59.1±3.00##
4	Test	89.1±2.38	54.1±3.27#

All values are expressed in mean ± SEM n=6.*** indicates p<0.001 when disease control group compared with normal group### indicates p<0.001, ## indicates p<0.01, # indicates p<0.05 when standard and test groups are compared with disease control group.

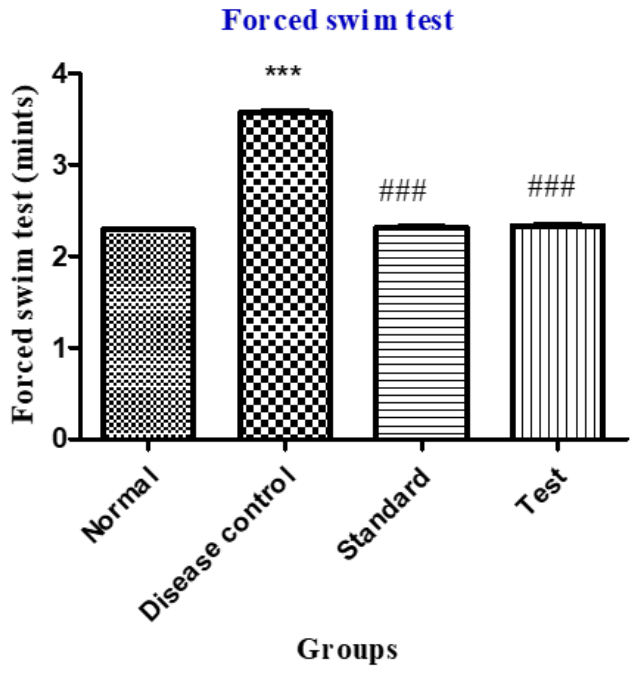

Figure 4: Effect of spirulina on duration of immobility time taken to forced swim test.

RESULTS

Effect of spirulina on *in vivo* antioxidant parameters

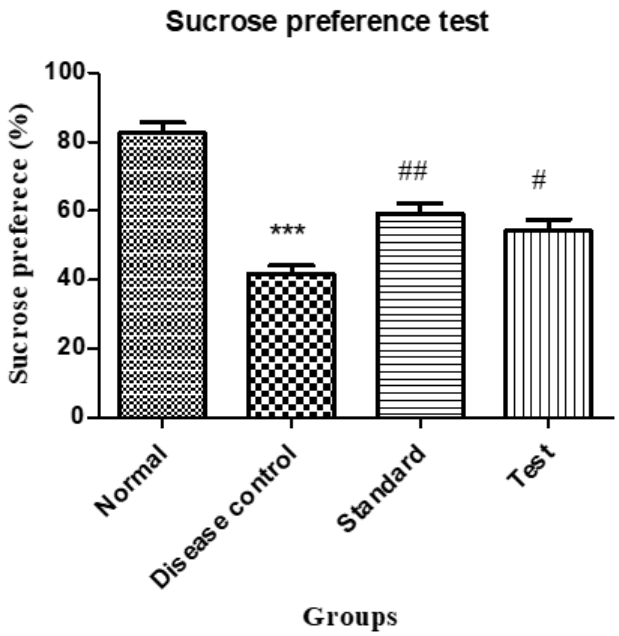

Figure 5: Effect of spirulina on sucrose preference test.

Where,

A: Initial absorbance.

B: Final absorbance (after 30 sec).

Units: μ moles of H_2O_2 consumed/mg protein/min in brain.

Table 9: Effect of spirulina on *in vivo* anti-oxidants.

Groups	SOD (µ/ mg protein)	CAT (µM H_2O_2 consumed / mg protein)	GSH (µg of GSH/mg protein)	LPO (nm of MDA/mg protein)
Normal	12.33±1.05	11.2±0.7	10.1±0.3	1.52±0.12
Disease control	3.55±0.31***	5.6±0.2***	3.43±0.1***	3.34±0.20***
Standard	7.95±0.30###	8.9±0.14###	7.27±0.42###	1.34±0.08###
Test	6.75±0.2##	7.7±0.2##	6.71±0.26###	1.61±0.03###

All values are expressed in mean ± SEM.*** indicates $p<0.001$ when disease control group compared with normal group.### indicates $p<0.001$, ## indicates $p<0.01$ when standard and test groups are compared with disease control group.

DISCUSSION

Effect of spirulina on forced swim test

The length of time that people in the disease control group stayed still after the forced swim test was significantly ($p<0.001$) longer than in the normal control group on the 7th, 14th, 21st, and 28th days, which is a sign of sadness in the disease control group. The length of time that the standard group stayed immobile after the forced swim test was significantly ($p<0.001$) shorter on the 7th, 14th, 21st, and 28th days than in the disease control group, which shows that fluoxetine has an antidepressant effect [Table 6]. The length of time that the test group stayed still after the forced swim test was significantly ($p<0.001$) shorter in the 7th, 14th, 21st, and 28th days compared to the disease control group, which shows that Spirulina has a calming effect. The length of time that the test group stayed still after the forced swim test didn't vary significantly ($p<0.001$) from the standard group, which was given 10 mg/kg of fluoxetine. This shows that Spirulina and fluoxetine have almost the same antidepressant effect.

Effect of spirulina on duration of immobility tail suspension test

On the 7th, 14th, 21st, and 28th days, people in the disease control group stayed still longer after the forced swim test than people in the normal control group. This is a sign that people in the disease control group are sad. After the forced swim test, the standard group stayed still for significantly ($p<0.001$) less time than the disease control group on the 7th, 14th, 21st, and 28th days [Table 7]. This shows that fluoxetine has an antidepressant effect. After the forced swim test, the test group stayed still for significantly ($p<0.001$) less time than the disease control group on the 7th, 14th, 21st, and 28th days. This shows that Spirulina has a cooling effect. After the forced swim test, the test group didn't stay still much longer ($p<0.001$) than the normal group, which was given 10 mg/kg of fluoxetine. This shows that the depressive effects of Spirulina and fluoxetine are almost the same [Figure 4]. (400 mg/kg) had significantly shorter periods of immobility after the tail suspension test (7th, 14th, 21st, and 28th), which is evidence of the antidepressant effect of Spirulina. The length of immobility following the tail suspension test did not differ significantly ($p<0.001$) between the test group receiving 400 mg/kg of spirulina and the control group receiving 10 mg/kg of fluoxetine, suggesting that the two supplements have roughly equivalent antidepressant effects.

Effect of spirulina on sucrose preference test

Compared to the group that didn't get spirulina, the group that got 400 mg/kg of spirulina had a significant ($p<0.05$) increase in sucrose consumption on the 28th day. When compared to the usual group, the disease control group's consumption of sucrose dropped significantly ($p<0.001$) on day 28. This showed that the CUS model caused stress. Compared to the disease control group, the standard group that was given 10 mg/kg of fluoxetine had a significant ($p<0.01$) increase in the amount of sugar they ate on day 28. Effect of spirulina on Superoxide Dismutase (SOD) levels [Table 8].

When comparing the illness control group to the normal group, the SOD levels in the brain sample taken after the 29th day showed a striking ($p<0.001$) decrease because of the overproduction of free radicals [Figure 5]. When compared to the illness control group, fluoxetine treatment groups showed a significant ($p<0.001$) rise in SOD levels, while test drug treatment groups showed a substantial ($p<0.01$) increase in SOD levels, demonstrating the anti-oxidant action of spirulina.

Effect of spirulina on Catalase (CAT) levels

It was discovered that the increased production of free radicals significantly ($p<0.001$) lowered the CAT levels in the disease control group compared to the normal group in the brain sample collected after the 29th day. The fluoxetine treatment groups showed a significant ($p<0.001$) increase in CAT levels when compared to the illness control group, whereas the test drug treatment groups displayed a substantial ($p<0.01$) increase in CAT levels, demonstrating the anti-oxidant action of spirulina [Table 9].

Effect of spirulina on reduced glutathione (GSH) levels

The increased generation of free radicals was reported to have significantly ($p<0.001$) reduced the GSH levels in the disease control group when compared to the normal group in the

brain sample obtained after the 29th day. When compared to the ischemia control group, both the standard treatment group and the test drug treatment group showed a substantial ($p<0.001$) increase in GSH levels, demonstrating spirulina's antioxidant activity.

Effect of HAEPN on lipid peroxidation (LPO) levels

The MDA levels in the disease control group were significantly higher ($p<0.001$) than those in the normal group when compared to the LPO levels in the brain sample obtained after the 29th day. When compared to the disease control group, both the standard treatment group and the test drug treatment group showed a substantial ($p<0.001$) decline in MDA levels.

CONCLUSION

In the current study, antioxidant enzymes like SOD, CAT, and GSH were less active in the CUS-induced disease control group, while LPO levels went up by a large amount. That proves without a doubt that the overproduction of free radicals and the onset of the lipid peroxidation system are what cause tissue damage. In the groups that were given CUS, however, antioxidant enzyme activities like SOD, CAT, and GSH went up, while LPO levels went down by a lot. In this study, the depressive effects of spirulina algae were similar to those of the drug fluoxetine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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