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RESEARCH ARTICLE

Antidepressant Effects of *Cleistocalyx nervosum* Extract Consumption in a Rat Model of Post Stroke Depression

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Abstract

The aim of this study was designed to evaluate whether *Cleistocalyx nervosum* (*C. nervosum*) extract improved depression-like behaviors induced by middle cerebral artery occlusion (MCAO) in post-stroke-depression (PSD) rats. Right MCAO procedure was performed on day 1 in male Wistar rats. *C. nervosum* was thereafter administered (250, 500 and 750 mg/kg), daily for 28 days. Neurological functions after ischemia were determined by the forelimb grip force and the hot plate tests. Tail suspension (TST) and forced swimming (FST) tests were used to assess depression-like symptoms. Plasma corticosterone (CORT) level, and brain oxidative status, and scavenging enzymes were also investigated. Our results showed that three doses of *C. nervosum* treatment were able to diminish neurological impairments and exert antidepressant effects in PSD rats. This was accompanied by the decrease in stress hormone level and brain oxidation indices, and increase in antioxidant enzymes activities. It suggested that *C. nervosum* could improve the depression-like emotional status and promote recovery of motor and sensory functions in PSD, and can be applied as a complementary agent for treatment of PSD.

Keywords: *Post-stroke-depression, Cleistocalyx nervosum, Oxidative stress, Antidepressant, Stroke.*

Introduction

Depression symptom resulting from ischemic stroke is called post-stroke depression (PSD) and is considered the most frequently neuropsychiatric problem after cerebral ischemia [1]. PSD seriously affects the quality of life, limited recovery and rehabilitation in stroke patients, as well as increased cost of medical treatment, burden of family caregiver, and rate of mortality [2].

Similar to ischemic stroke pathophysiology, there is conclusive evidence suggests that oxidative stress plays a vital role in the pathophysiology of PSD [3]. A growing body of evidence shows abnormalities in the levels of lipid peroxidation products and antioxidant enzyme activity such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) occur in PSD patients [4-6]. Accumulating evidence implicate the dysregulation of hypothalamic-pituitary-adrenal (HPA) axis in the pathophysiology of depression [7].

Some reports found significantly higher cortisol in individuals with major depression [8]. It has been reported that a patient who has a depressive disorder with elevated cortisol concentration are less responsive to psychotherapy treatments, implying that medications that may reduce cortisol concentration, may be a significant part of a depression treatment regimen for patients with depression [9].

At present, the mainstay of therapy for PSD patients is based on treatment with antidepressants, which may cause various and serious adverse side effects such as nausea, constipation, arrhythmias, orthostatic hypotension, cholinergic deficits, weight gain and sexual dysfunction [10]. It is important to search for novel agents or new compounds of PSD treatment that could increase conventional therapies. In this context, natural products have a unique

chemical variety, which lead to biological activities, and drug-like properties [11]. Thus, natural products such as fruits or plants have been considered as an option for the development of new drugs for treating human diseases, including PSD.

Cleistocalyx nervosum var. *paniala* (*C. nervosum*), is widely grown in Southeast Asia, especially in the northern region of Thailand [12]. This plant belonging to Myrtaceae family. Several reports reveal the various medicinal activities and health benefits of *C. nervosum* fruit such as anti-carcinogenic, anti-heavy metal toxicity, antimicrobial activities, and antioxidant properties [13-15].

In addition, our group has previously revealed the neuroprotective effects of *C. nervosum* against focal cerebral ischemia in rats [16]. As information mentioned above and increasing interest in the use of natural products on human disease prevention led us to explore the antidepressant activity of *C. nervosum* extract consumption in a rat model of PSD.

Materials and Methods

Chemicals and Reagents

Nicotinamide adenine dinucleotide phosphate (NADPH), 5,5'-dithiobis-2-nitrobenzoic acid, 2, 2-diphenyl-1-picrylhydracyl (DPPH) were obtained from the Sigma Chemical Company (St. Louis, USA). Potassium phosphate (K3PO4) and sodium chloride (NaCl) were obtained from Merck (Darmstadt, Germany). Ascorbic acid, chloral hydrate, and fluoxetine hydrochloride as a positive control drug were purchased from Siam Pharmaceuticals Ltd., Thailand. The other chemicals were of analytical grade and obtained from standard commercial.

C. Nervosum Extract Preparation

Fresh ripe *C. nervosum* fruits were collected from the garden at Chiangmai horticulture research center, Tambol Choeng Doi, Amphur Doi Saket Chiang Mai, Thailand, in August. All WP fruits were chosen for the absence of pests, defects and diseases, and then had their peels were removed and washed carefully with running tap water, and dried with hot air oven at 40°C for 24 h. The dried materials were blended and extracted with distilled water then filtered and freeze-dried in lyophilizer, and the end product yield of 11.33%. The WP peel extract was stored in light-protected bottle at -20°C until use and all dilutions of this extract were dissolved in distilled water.

Animals and Experimental Groups

Young male Sprague-Dawley rats (200-250 g) from Nomura Siam International Co, Ltd., based in Bangkok, Thailand, were used in the current study. All rats, maintained in a standard cages, were kept at constant room temperature (20±2°C) with a 12 h light/dark cycle (light from 7:00 a.m. to 7:00 p.m.) and had free access to a pellet diet and tap water.

These animals were allowed to acclimatize to the new environment for 7 days before to use in experiments. The study was conducted on the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995)" and all procedures involving animals in this study were performed in accordance with the approval of the Institutional Animal Care and Use Committee of University of Phayao, Thailand (No. 5701040009).

The rats were allocated randomly into six groups (n=8/group) as follows: Sham treated group daily orally with distilled water (Sham), PSD treated group (PSD), PSD + fluoxetine 20 mg/kg treated group which serve as a positive control (PSD + fluoxetine), PSD + *C. nervosum* 250 mg/kg treated group (PSD + *C. nervosum* 250), PSD + *C. nervosum* 500 mg/kg treated group (PSD + *C. nervosum* 500), and PSD + *C. nervosum* 750 mg/kg treated group (PSD + *C. nervosum* 750).

Rats in all groups, except for those in a sham group, underwent permanent right middle cerebral artery occlusion (MCAO) to induce PSD. On the 2nd day after MCAO, all rats were orally given the assigned substances as mentioned earlier for 28 days. The evaluation of neurological deficit scores and depressive-like behaviors were performed every 14 days after MCAO. On last day, after the behavioral assessments, all rats were scarified to evaluate the biochemical, the oxidative status and the antioxidant effects of *C. nervosum* in PSD model.

PSD-Model Induction

PSD was developed by the MCAO method as described previously, with slight modifications [17].

Following overnight fast, rats were anesthetized with intra-peritoneal (i.p.) injection of 10% chloral hydrate (40 mg/kg) and placed on a heating pad in supine position. The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA), were exposed and isolated through a neck midline incision. A 4-0 monofilament nylon was introduced from the right ECA into the lumen of the ICA to occlude the right MCA. Sham treated group underwent the same surgical procedure, but without the nylon filament insertion. After MCAO operation, rats were allowed to recover for 2 days prior to the start of any intervention.

Neurological Determination in Rats

Forelimb Grip Force Test

The grip strength test is a simple test to assess the muscle strength of rodents. A grip strength meter (model MK-380Si, Muromachi Kikai Co., Ltd., Japan) was used in this study. In this case, the rat was allowed to grasp the tension bar of grip apparatus and were then pulled backwards in the horizontal plane until it could no longer hold onto the bar. The peak force was recorded automatically in grams.

Hot Plate Test

The hot plate test is a method to evaluate the sensory function recovery of rodents. Placing a rat on a heated floor of hot plate apparatus which was maintained at 50°C. The latency of foot withdrawal reflex in response to heat stimuli was recorded and used as the index of the sensory function recovery exposed to heat stimuli.

Determination of Depressive-like Behavior in Rats

The Tail Suspension Test (TST)

The TST is commonly model used to evaluate the depressive-like behavior in rodents [18]. 30 min after oral administration of all substances, rat was suspended by its tail from the ceiling of the apparatus with adhesive tape. The approximate distance between the rat's tail tip and the apparatus floor was 30 cm. Immobility time was recorded when the rat stops struggling and hangs immobile posture, was considered to have "given up" which is the characteristic of a depressive-like stage.

The Forced Swimming Test (FST)

The FST was performed as previously described Porsolt et al [19] with slight modifications. Rats were individually placed for 5 min in a glass cylinder (H: 45 cm, D: 25 cm) containing 25 cm fresh water at 25°C. The immobility time (floated in the water without struggling and neither hind leg was minor moving to keep its head above the surface of water) was recorded by using a stopwatch.

Biochemical Parameter, Antioxidant Capacity and Oxidative Status Measurement in Rats

All rats were quickly decapitated, and blood sample were rapidly collected in a plain bottle, centrifuged at 4000 x g for 15 min, and serum was collected in Eppendorf tubes to determine the corticosterone (CORT) concentration, whereas the brain of each rat was collected to measure the scavenging enzyme activity and malondialdehyde (MDA) level, a product of lipid peroxidation.

Determination of Serum CORT

The CORT concentration was detected using the sandwich ELISA technique according to the manufacturer's protocol (Novus Biologicals, USA). The optical density (OD) was assess using a Versamax microplate reader (Molecular Device, LLC, USA) and read the OD at 450 nm.

Determination of SOD Activity

Amount of SOD activity in rat brain tissues was estimated according to the method of Kakkar et al [20]. The mixture contained 0.3 ml of supernatant, 1.2 ml of sodium pyrophosphate buffer (pH 7.0; 0.052 M), 0.1 ml of phenazine methosulphate (186 µM) after centrifugation (10,000 g for 30 min) of homogenate was added to the reaction mixture. The enzyme reaction started by adding 0.2 ml of NADH (780 µmol) and stopped after adding 1 ml of glacial acetic acid, and read the OD at 560 nm. SOD activity was expressed as U/mg protein.

Determination of CAT Activity

The method of Chance and Maehly [21] with slight modification was used to determine CAT activity. Briefly, a reaction mixture consisted of 0.1 ml of 10% brain tissue, 0.4 ml of 5.9 mM H_2O_2, 2.5 ml of 50 mM phosphate

buffer and deionized water. After 2 min incubation, the absorbance of the reaction mixture was read at 240 nm. CAT activity was expressed as U/mg protein.

Determination of MDA Level

MDA level in rat's brain was measured as described Okhawa et al [22]) with some modifications. In brief, rat brain tissues were homogenized in Tris-HCl buffer solution (pH 7), added with 2 mL of thiobarbituric acid (TBA) reagent, and heated at 100° C for 30 min. Added 5 ml of n-butanol-pyridine (15:1) and 1 ml of distilled water in the mixture and centrifuged at 4000 rpm for 10 min at 4 °C. The OD of the supernatant was read at 532 nm. The MDA concentration was expressed as µmol/g of protein. Total protein in the brain tissue homogenate was determined according to the method of Lowry et al [23].

Statistical Analysis

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All statistical calculations were performed using SPSS software version 17.0. All data were expressed as the mean ± standard error of mean (SEM) and were analyzed using the one-way ANOVA followed with Tukey's *post-hoc* test. A value of $p < 0.05$ was accepted as significance.

Results

Effects of *C. Nervosum* on motor and Sensory Functions of PSD Rats

A significant decrease ($p < 0.05$) in grid force (g) exerted by both forepaws was noted in PSD group when compared to sham group. Compared with the PSD group, oral administration of *C. nervosum* (250 mg/kg) and *C. nervosum* (500 mg/kg) significantly increased the force in PSD rats ($P < 0.05$) on the 28th day. However, *C. nervosum* (750 mg/kg) initially showed increased grid force since 14th day (Figure 1).

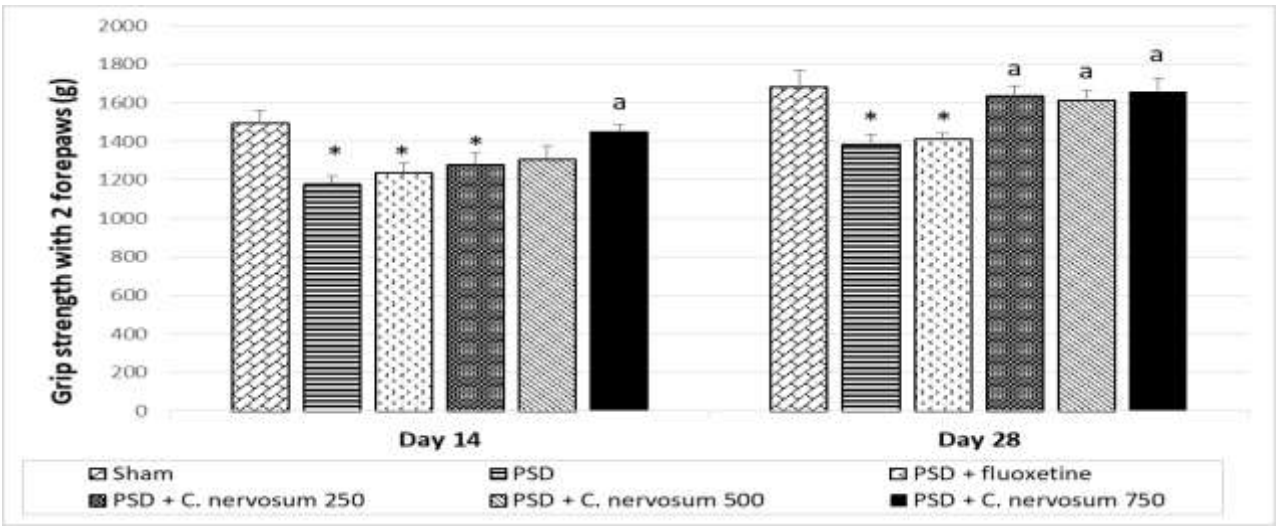

Figure 1: Effects of the *C. nervosum* on force exerted with both forepaws of PSD rats. Data are expressed as mean ± SEM. (n=8), *p < 0.05 compared with the sham group. [a]p < 0.05 compared with the PSD group

As shown in Table 1. The foot withdrawal time from hot plate test in the PSD group was significantly higher than in the sham group ($p < 0.05$).

All various doses of *C. nervosum* treatment significantly decreased foot withdrawal time in the PSD rats ($p < 0.05$).

Table 1: Effect of *C. nervosum* extract on foot withdrawal time in hot plate test of PSD rats

Treatment	Foot withdrawal time (seconds)	
	Days after MCAO	
	14th day	28th day
Sham	1.82 ± 0.15	1.80 ± 0.18
PSD	4.38 ± 0.36*	4.14 ± 0.27*
PSD + fluoxetine	3.88 ± 0.25*	3.42 ± 0.31*
PSD + *C. nervosum* 250	3.97 ± 0.42*	2.54 ± 0.10[a]
PSD + *C. nervosum* 500	3.99 ± 0.32*	2.51 ± 0.12[a]
PSD + *C. nervosum* 750	3.54 ± 0.15*	2.02 ± 0.20[a]

Data are expressed as mean ± SEM. (n=8), *p < 0.05 compared with the sham group. [a]p < 0.05 compared with the PSD group.

Antidepressant Effects of *C. Nervosum* in PSD Rats

14th and 28th day following the beginning of all substances in PSD rats, the tail suspension test presented that the rats in PSD group show significantly ($p < 0.01$) longer mean immobility time as compared to those of sham group on all the evaluation days (Figure 2). However, three doses of *C. nervosum* showed significantly ($p < 0.05$) reduction in immobility time as compared with the PSD group. However, it is noted that only *C. nervosum* at dose of 750 mg/kg showed significantly ($p < 0.05$) shorter mean immobility time since 14th day similar effect to those of fluoxetine, used as antidepressant drug.

Figure. 3 showed that the longest immobility time duration was observed in the PSD group compared with the sham group ($p < 0.01$). Compared with the PSD group, treatment of fluoxetine significantly decreased the immobility time in PSD rats ($P < 0.01$). Similarly, immobility time also significantly reduced in both of *C. nervosum* (250, 500 mg/kg) and *C. nervosum* (750 mg/kg) groups compared with the PSD group ($p < 0.05$ and $p < 0.01$ respectively).

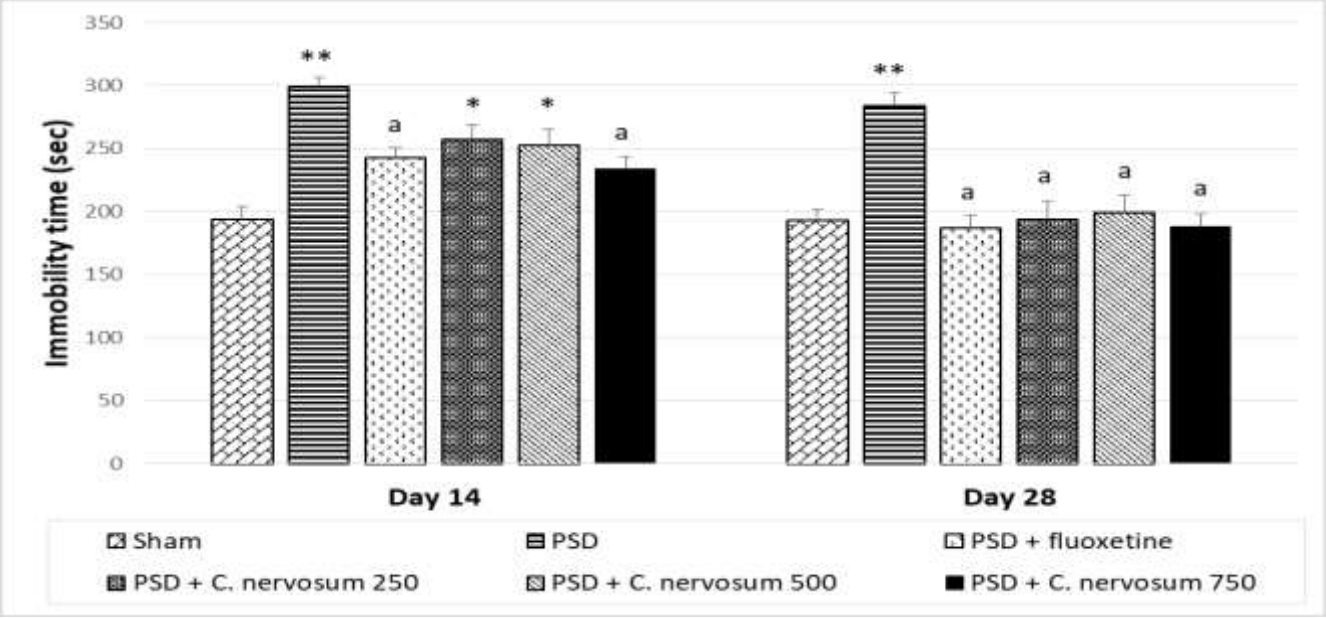

Figure 2: Effects of the *C. nervosum* on the immobility time in tail suspension test of PSD rats. Data are expressed as mean ± SEM. (n=8), *p < 0.05, **p < 0.01 compared with the sham group. [a]p < 0.05 compared with the PSD group

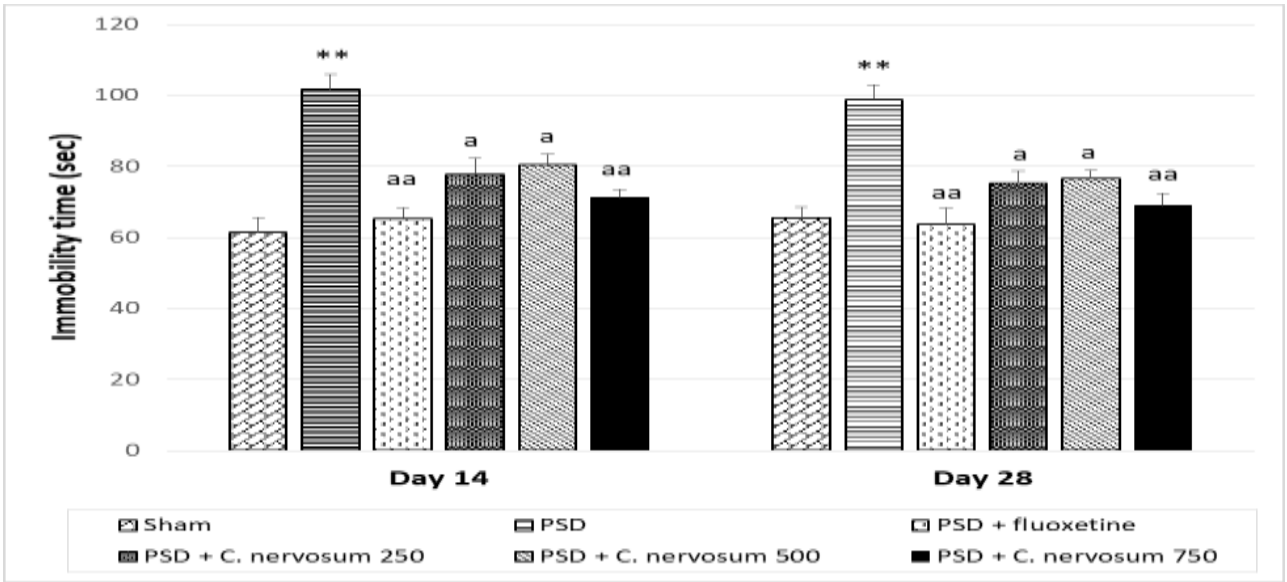

Figure 3: Effects of the *C. nervosum* on the immobility time in FST of PSD rats. Data are expressed as mean ± SEM. (n=8), **p < 0.01 compared with the sham group. [a]p < 0.05, [aa]p < 0.01 compared with the PSD group

Effect of *C. Nervosum* on CORT Concentration of PSD Rats

Figure 4 shows the plasma CORT concentrations in all groups. Compared with the sham group, indicated that PSD significantly induced plasma CORT ($p < 0.01$). *C. nervosum* at doses 250 and 500 mg/kg ($p < 0.05$) whereas *C. nervosum* at doses 750 mg/kg and fluoxetine ($p < 0.01$) significantly reduced plasma CORT concentrations to normal levels.

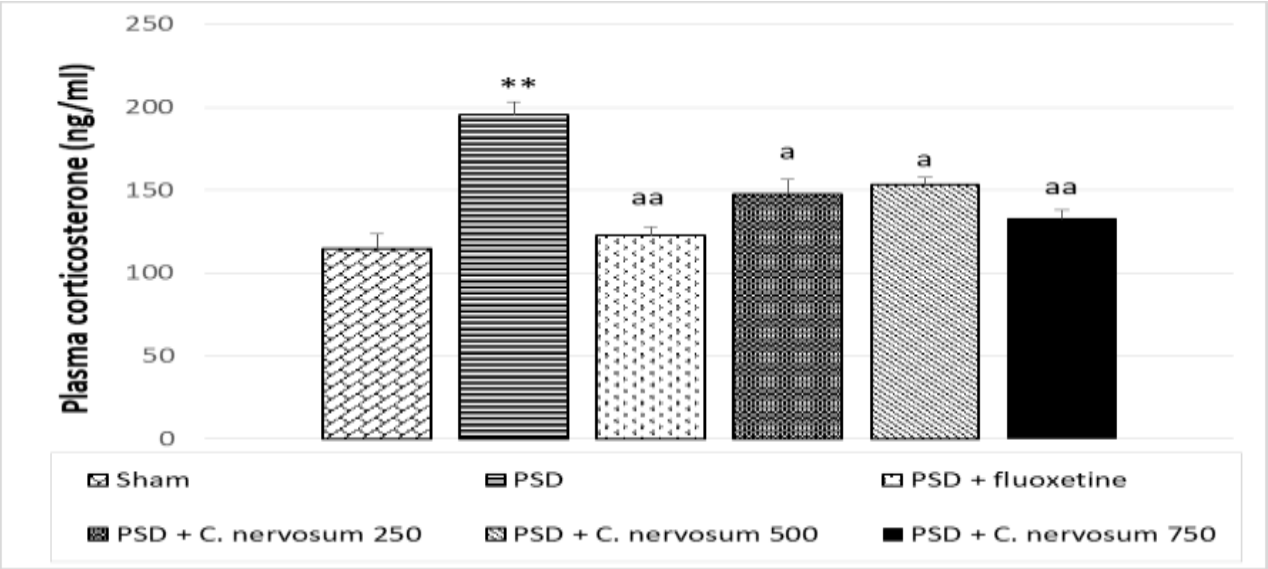

Figure 4: Effects of the *C. nervosum* on the plasma CORT of PSD rats. Data are expressed as mean ± SEM. (n=8), **p < 0.01 compared with the sham group. [a]p < 0.05, [aa]p < 0.01 compared with the PSD group

Oxidative Status and Antioxidant Effects of *C. nervosum* in PSD Rats

As shown in Table 2. There was a significant difference between the sham and PSD groups with regard to the brain expression of MDA level ($p < 0.05$); in contrast, SOD and CAT activities reduced significantly ($p < 0.05$), which indicated that oxidative stress occurred. Compared with the PSD group, brain MDA level in the all doses of *C. nervosum* groups were significantly decreased (all $p < 0.05$), and restored SOD and CAT activities ($p < 0.05$).

Table 2: Effects of the *C. nervosum* on the MDA level, SOD, and CAT activities in the brain of PSD rats

Treatment	MDA (µmol/g prot)	SOD (U/mg prot)	CAT (U/mg prot)
Sham	3.82 ± 0.72	133.14 ± 16.81	7.52 ± 0.42
PSD	6.64 ± 0.51*	93.26 ± 20.63*	3.41 ± 0.53*
PSD + fluoxetine	6.26 ± 0.73*	95.77 ± 21.34*	3.65 ± 0.29*
PSD + *C. nervosum* 250	4.16 ± 0.52[a]	120.45 ± 17.21[a]	5.77 ± 0.35[a]
PSD + *C. nervosum* 500	4.08 ± 0.38[a]	121.22 ± 20.13[a]	6.12 ± 0.46[a]
PSD + *C. nervosum* 750	3.97 ± 0.32[a]	127.66 ± 15.85[a]	6.65 ± 0.34[a]

Data are expressed as mean ± SEM. (n=8), *p < 0.05 compared with the sham group. [a]p < 0.05 compared with the PSD group.

Discussion

Several studies have reported that the biological mechanisms dysfunction is one of the important factor in the neuropathogenesis of PSD. These include the hypothalamic-pituitary-adrenal (HPA) axis dysfunction, oxidative stress, and brain neurotransmitter abnormalities [24]. Due to the limitation of the current treatment for PSD, and the diverse pharmacological actions and display strong antioxidant activity of *C. nervosum*. This study aims at investigate the impact of the effects of *C. nervosum* extract consumption in a rat model of PSD. Our recent study has presented, for the first time the antidepressant effects of the extract from *C. nervosum* consumption for 28 days could ameliorated the neurological impairments, and change in biological indices and behavioral patterns of PSD in rats. PSD patients often feel useless and depress when they are unable to accomplish tasks that were previously easy and living conditions

become difficult due to their disability [25]. Previous studies have shown that a PSD model was established through MCAO surgical method, to imitate the neurological and behavioral abnormalities of PSD [26-27], which is consistent with our current work. Our results revealed that MCAO induced cerebral ischemia led to motor and sensory dysfunctions, and increased immobility representative the degree of despair in new stimulating situations, which indicated that MCAO method was more effective in inducing PSD.

Forelimb grip force test is a widely used method to evaluate the motor function [28], and the hot plate test is a test of the pain sensation [29]. The evaluation of animal behavior showed that PSD rats had impairments of motor and sensory functions. All various doses of *C. nervosum* extract treatment increased grid force and reduced the foot withdrawal time in PSD rats, which presented significant differences between the PSD and PSD + *C. nervosum* groups.

TST and FST paradigms are widely accepted behavioral tests for determine pharmacological antidepressant activity [30], [31]. We noticed that there were significant changes, notably an increase in immobility both in TST and FST paradigms of PSD rats, which is similar to the behavioral changes observed in depressed human patients.

After oral administration of *C. nervosum* or fluoxetine for 28 days attenuated the depression like behavior of PSD rats, which is evident in a reduction of immobility time in rats subjected to the TST and FST. Therefore, our data show that these depression symptoms in PSD rats were improved by *C. nervosum*, suggesting that this extract has antidepressant-like effects, similar to the effects of fluoxetine. However, it is noted that fluoxetine treatment did not improve the motor and sensory functions in PSD.

Strong evidence from several studies reveal that oxidative stress is involved in the pathogenesis of PSD [32-33]. Depression commonly occurs in neurodegenerative disorders, such as stroke, and is accompanied by a decrease in antioxidant status and an induction of oxidative damage caused by free radicals [34].

MDA is the end product of lipid peroxidation that can be used as a marker for oxidative damage. Results of the current study showed that PSD rats exhibited significant higher MDA level in the brain than the sham rats. This data is in agreement with Liu et al [35], who presented brain MDA level higher in PSD patients. Decline in brain antioxidant enzymes activities in the postmortem brain from depression patients has been well documented [36].

Similarly, in our study, significant lower activities of SOD and CAT enzymes were observed in the PSD rats. For this reason, many other substances exerted antioxidant effects have been proposed as targets for novel antidepressants [37]. Interestingly, our data demonstrated that all doses of *C. nervosum* extract supplementation was able to decrease MDA concentration and restore both SOD and CAT levels back to normal, signifying its antioxidant activity attenuate PSD like behaviors.

A large number of studies have looked at relationships between depression and HPA axis dysregulation [38]. Data show that plasma cortisol is higher in depression patients [39]. In this study, an increase in plasma CORT level was observed in PSD rats. Here, *C. nervosum* extract administration decreased plasma CORT level, suggesting that the *C. nervosum* reduced HPA axis activity in PSD.

Taken all results considerations, the possible mechanisms of antidepressant effects of *C. nervosum* may occur via its antioxidant effects and decreased activity of the HPA axis. As regards the limitations of this study, the exact mechanism and isolation of active components of *C. nervosum* exhibits antidepressant like activity were not be measured here. Therefore, future studies should investigate to further ensure the action mechanisms of this extract on antidepressant effects.

Conclusion

The current study reports that, *C. nervosum* extract consumption attenuated neurological impairments and improved depression symptoms as proven by behavioral tests. Additionally, increased the antioxidant enzymes, decreased brain oxidative damage,

and normalization of the HPA axis (reduction in plasma CORT concentration) were observed. Therefore, *C. nervosum* extract may be a novel agent in preventing or treating PSD.

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