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Original Research Article (Experimental)

Effect of *Coelogyne cristata* Lindley in alleviation of chronic fatigue syndrome in aged Wistar rats

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ABSTRACT

Background: *Swarna Jibanti* scientifically known as *Coelogyne cristata* Lindley (Orchidaceae), an orchid mentioned in Ayurvedic medicine is used to promote healthy life span.
Objective(s): The present work was planned to study the efficacy of hydro-alcoholic extract of pseudo-bulbs of *C. cristata* (CCE) to assess its role on chronic fatigue syndrome (CFS) induced behavioural and biochemical changes in aged Wistar rats compared to *Panax ginseng* (PG), a prototype anti-stress agent.
Materials and methods: CFS was induced by forced swimming for consecutive 21 days for fixed duration (15 min sessions). The criteria of CFS due to fatigue were counted using locomotor activity, depression and anxiety through automated photactometer, immobility time and plus maze activity respectively. Acute toxicity study of CCE (upto 2 g/kg, Limit test) was also performed. For CFS, animals were divided into five groups, naive control, control, CCE treated (25 mg/kg b.w., 250 mg/kg b.w.) and standard PG treated (100 mg/kg b.w.) groups. All drugs were given orally for consecutive 21 days along with CFS. After assessing behavioural parameters, all animals were sacrificed at day 21 and *in vivo* antioxidant potential of CCE was determined by lipid peroxides, nitrite, catalase (CAT) and superoxide dismutase (SOD) in brain tissue.
Results: CCE was found to be non-toxic. CCE treated aged rats significantly improved ($p < 0.001$) the spontaneous locomotor movement with respect to control rats, while, decreased the mobility period or depression score. In CFS, CCE also enhanced the time spent ($p < 0.001$) in open arms while reducing the time spent in closed arm as compared to CFS control, indicating lowering anxiety score. Moreover, marked diminution in lipid peroxidation, nitrite and SOD level was exhibited after CCE treatment and significantly enhanced catalase level significantly ($p < 0.01$) with respect to CFS control. PG also showed similar actions.
Conclusion: The results confirmed the potential therapeutic actions of CCE against experimentally induced CFS in aged rats that might be due to its CNS mediatory antioxidant properties.

1. Introduction

Coelogyne cristata Lindley (family: Orchidaceae) is a species of orchid, inhabitant in high altitudes (1600–2000 m) eastern Himalayan and famous as *Swarna Jibanti* in West Bengal, India and also in Bangladesh [1,2]. *Swarna Jibanti* is used as a stimulant and tonic in aged patients suffering from persistent diseases, like asthma, degenerative changes and blood borne diseases [3–6]. In Ayurveda, it is included under the *Vayasthāpaka* or anti-ageing drug [7–9]. Chemical analyses revealed the presence of two phenanthrenes, *coeloginanthridin* (*3,5,7-trihydroxy-1,2-dimethoxy-9,10-dihydrophenanthrene)* and *coeloginanthrin* (*3,5,7-trihydroxy-1,2-dimethoxyphenanthrene*) [10,11]. Further investigation afforded two new stilbenoids, designated *coeloginone* and *coeloginanthrone* [12]. Phenanthrenes are the prototypical opioids which are presumably formed by oxidative coupling of the aromatic rings of

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stilbene precursors and possess several biological activities [13]. Phenanthrenes have been studied for cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, anti-platelet aggression, anti-allergic, immunomodulatory, anticancer, anti-aging, atherosclerosis properties [13–15]. The anti-stress and antioxidant activity of similar herb from Orchidaceae family have also been reported [16,17].

Though this orchid is traditionally used in geriatric patients in Indian subcontinent, but there is no scientific data available. However, the biggest challenge with geriatric problem is that in most of the cases the condition cannot be attributed to a single cause or in certain conditions like neuropsychiatric disorders [18]. A number of studies in humans and experimental animals provide evidence that hyperactivity of the HPA (hypothalamic pituitary adrenal) axis contributes to neuronal and peripheral deterioration associated with aging [19,20]. There is clear evidence that increase in glucocorticoidal activity and central CRH or corticotropin releasing hormone during aging can have damaging effects and contribute to pathological conditions associated with advancing age such as depression, anxiety, neurodegeneration, immune and metabolic disorders [18,21]. On the other hand, chronic fatigue syndrome (CFS) is mainly characterized by prolonged disabling fatigue of underlying psychiatric and neurological disorders requiring appropriate psychiatric, psychological and neurological evaluation [22–24]. It is well established that there is a high lifetime prevalence of affective symptoms, such as depression, dysthymia, and anxiety in CFS [25–27]. There are strong correlations between oxidative damage in brain due to CFS and aging process [25,28–30].

The present study was designed to explore the effectiveness of phenanthrene rich extract of orchid *C. cristata* Lindley in CFS induced behavioural changes in aged animals. Biochemical estimations were also carried out to establish the antioxidant activity of this plant *in vivo* whereas *Panax ginseng* was used as prototype standard [25,31,32].

2. Materials and methods

2.1. Drug preparation

The pseudobulbs of *C. cristata* Lindley were procured from the local drug market of Kolkata, India and authenticated by Department of Botany, Burdwan University, West Bengal; voucher specimen was deposited in the museum of the Department of Pharmacognosy, National Research Institute of Ayurvedic Drug Development, Kolkata (NRIADD/2011/03). The plant materials were cleaned and dried in shed and a coarse powder was prepared with the help of pulveriser. The sieved coarse material was successively defatted with petroleum ether and chloroform and then extracted with hydro-alcohol (60%) for 72 h. The extract was concentrated under reduced pressure to obtained dry mass (CCE). Hydro-alcoholic extract was further purified by several silica chromatographics and the phenanthrenes analogue was identified by HPTLC, HPLC and LC-MS analysis [33].

2.2. Animals

Inbred male Wistar rats of 20 months (300–325 g) were selected based on body weight and grouped for pharmacological evaluation. Animals were acclimatized for 7 days and health examination was performed during acclimatization period. Rats were housed individually in polypropylene cages, fed animal pellet diet, mineral water *ad libitum* during the entire study period. The temperature was maintained at 22 ± 2 °C along with relative humidity of 60–70% and illumination was controlled to give approximately a sequence of 12 h light and 12 h dark. The animal experiments were conducted in accordance with the standard ethical guidelines of the Institutional Animal Ethics Committee (Approval No: IAEC/2010/7-09, dated 10/03/2010).

2.3. Acute toxicity study

CCE was administered at different doses (50, 100, 200, 400, 800, 1600 & 2000 mg/kg p.o) in arithmetical progressive manner to normal rats to investigate the lethal dose of plant extract [34]. The animals were observed carefully for signs of toxicity, morphological, behavioral differences and mortality during first 4 h, and then kept under observation for next 14 days. Rats receiving different doses of CCE did not manifest any clinical signs of toxicity up to dose level of 2 g/kg body weight per oral and there was no mortality. Further higher doses could not be administered due to stickiness and less solubility of the material.

2.4. Experimental protocols

Aged male Wistar rats were divided into five groups (n = 6). After doing pilot experiments, two oral doses of CCE *i.e.* 125 mg/kg b.w. and 250 mg/kg were finally selected for the study and *P. ginseng* 100 mg/kg b.w. (p.o) was used as prototype standard [27,31,33]. The animals were grouped as follows:

Group I: Naïve animals, which were neither subjected to stress nor given any extract.

Group II: Control animals, subjected to forced swimming (to induce CFS) for 21 days, but without given any extract; received only vehicle (distilled water, 0.5 ml/100 g).

Group III: Test animals (CCE-125), subjected to forced swimming and treated with CCE at the dose of 125 mg/kg b.w. for 21 days.

Group IV: Test animals (CCE-250), subjected to forced swimming and treated with CCE at the dose of 225 mg/kg b.w. for 21 days.

Group V: Test animals (PG-100), subjected to forced swimming and treated with *P. ginseng* at the dose of 100 mg/kg b.w. for 21 days.

C. cristata (CCE) and *P. ginseng* (PG) powdered extracts were administered orally each day, 1 h prior to exposure to forced swimming.

2.5. Chronic fatigue syndrome induced by swimming

The rats were exposed to forced swimming (except Group I) to induce chronic fatigue. Animals were forced to swim for 15 min sessions every day for 21 days, in glass bath tub (45 cm × 20 cm × 45 cm) containing water up to 30 cm height at room temperature (22–24 °C). After initial vigorous activity, each animal assumed a typical immobile posture intermittently with complete cessation of movements. After swimming for 10 min, the immobility period for next 5 min was observed on 21 consecutive days. Drugs were administered 1 h before the test on each day. The total duration of immobility period in seconds for the period of 10–15 min was noted for alternative every 7 days, up to 3 weeks. This chronic exposure of forced swimming produced depression and fatigue which represented chronic fatigue syndrome [30,36,37].

2.6. Locomotor activity

The somatomotor activity in aged rats was assessed using digital photo-actometer (Sentwin, India). Before subjected to CFS, individual rat was placed on the photo-actometer and its movements

Table 1
Effect of *C. cristata* extract and *P. ginseng* extract on locomotor activity in chronic fatigue aged rats.

Groups	Treatments	Photo-actometer score in 5 min			
		Day-1	Day-7	Day-14	Day-21
I	Naïve	162.4 ± 3.18	168.7 ± 4.06	160.7 ± 3.98	155.9 ± 3.15
II	CFS	162.9 ± 4.62[a]	129.4 ± 2.67[a***]	109.8 ± 3.87[a***]	82.6 ± 5.11[a***]
III	CFS+CCE-125 mg/kg	165.7 ± 4.59[b]	145.6 ± 3.72[b*]	125.1 ± 2.06[b]	120.5 ± 5.79[b***]
IV	CFS+CCE-250 mg/kg	160.8 ± 3.83[b]	141.6 ± 2.93[b*]	118.4 ± 2.19[b]	106.2 ± 4.57[b***]
V	CFS+PG-100 mg/kg	164.3 ± 3.94[b]	152.5 ± 3.64[b**]	137.6 ± 2.81[b*]	128.1 ± 5.38[b***]

For comparison between four groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., n = 6. Naïve group comprises vehicle treated animals who were not subjected to chronic forced swimming (CFS), control refers to vehicle treated group subjected to CFS, CEE refers to *C. cristata* extract and PG refers to *P. ginseng* extract. [a] as compared to naïve control and [b] when compared to chronic forced swimming control group of the same day. * represented $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

(digital score) was noted for 5 min. Modification of locomotor function was assessed on days-1, 7, 14 and 21 in all groups of rats prior to swimming [30,38].

2.7. Anxiety level

Elevated plus maze, is a unique test for evaluation of anxiety in rodents. The maze has two opposite open arms, 50 cm × 10 cm, crossed with two opposite enclosed arms of the same dimension with 40-cm high walls. The arms are connected by a central square, measuring 10 cm × 10 cm, giving the apparatus the shape of a plus sign. The whole maze is elevated to a height of 50 cm. The baseline anxiety levels of each animal was assessed 1 h after subjecting it to chronic forced swimming on days-1, 7, 14 and 21 [30,38]. The rats were placed individually at the centre of the plus maze with their head facing towards an open arm. During the next 5 min time period the following parameters were noted: total time spent in closed arm (in sec), total time spent in open arm (in sec) and total number of entries in open arm [39].

2.8. Biochemical estimation

After completion of all behavioral study, the animals were sacrificed by decapitation. The brains were removed and homogenized in 0.1M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation at 1000 ×*g* for 15 min, and for other assays, the homogenate was centrifuged at 10,000 ×*g* for 20 min, at 4 °C [27]. Thereafter, biochemical assessment of brain homogenate done in all groups of rats included following:

Protein estimation: Protein content in brain homogenate was measured according to the method of Lowry et al. (1951) using bovine serum albumin as standard [40].

Assessment of lipid peroxidation: The amount of lipid peroxidation and finally malondialdehyde (MDA) formation was estimated spectrophotometrically at 532 nm after reaction with thiobarbituric acid and expressed as nM/mg protein [41].

Nitrite assay: The amount of nitrite present in brain was estimated spectophotometrically using Greiss reagent [42].

Superoxide dismutase assay: Superoxide dismutase activity measured in brain tissue was according to method of Winterbourne using nitro blue tetrazolium dye (NBT) reduction method [43].

Estimation of catalase: Catalase activity was measured spectrophotometrically according to the method of Sinha (1972) [44].

2.9. Statistical analysis

All data were evaluated using descriptive and ordinal statistics. Results were expressed as mean ± SD. Comparison between control group and treated group was performed by multiple group comparison tests as Kruskal–Wallis test followed by Mann–Whitney *U*-test. In all the tests, criterion for statistical significance was $p < 0.05$.

3. Results

3.1. Chemical assay

Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, sterols in CCE. The successive extract of pseudobulb of *C. cristata* revealed the presence of active biomarkers through HPLC profiles. The HPLC chromatogram showed 12 peaks. However, one peak was prominent with significant percent area and height (> 67.97%). The most abundant peak with area and height was observed at the retention time 10.69 (R*t*, min), which is probably phenanthrene. Principal phenolic compounds were detected by liquid chromatography-mass spectrometry (LC-MS).

3.2. Acute toxicity study

All groups showed normal behaviour; no mortality and no abnormal symptoms, i.e. writhing, convulsion, hyper reactivity, hypothermia, lacrimation, salivation, fur or pilo-erection etc. during the study since 4 h of dosing up to 2 g/kg. There was no mortality observed upto 14 days.

Table 2
Effect of *C. cristata* extract and *P. ginseng* extract on depression score in chronic fatigue aged rats.

Groups	Treatments	Immobility score in 5 min			
		Day-1	Day-7	Day-14	Day-21
II	CFS	108.5 ± 2.23	146.4 ± 3.18	178.9 ± 3.46	194.7 ± 4.19
III	CFS+CCE-125 mg/kg	102.6 ± 2.18	118.7 ± 2.09***	127.4 ± 2.72***	138.5 ± 2.93***
IV	CFS+CCE-250 mg/kg	108.6 ± 1.72	114.5 ± 2.84***	121.8 ± 2.95***	129.2 ± 3.14***
V	CFS+PG-100 mg/kg	104.7 ± 1.95	126.1 ± 2.76**	131.7 ± 2.87***	142.6 ± 3.66***

For comparison between three groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., n = 6. Control group comprises vehicle treated animals who were subjected to chronic forced swimming (CFS), CEE refers to *C. cristata* extract and PG refers to *P. ginseng* extract. *represented $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared to chronic forced swimming control group of same day.

Table 3
Effect of *C. cristata* extract and *P. ginseng* extract on time spent in closed arm in plus maze in chronic fatigue aged rats.

Groups	Treatments	Closed arm time (sec)			
		Day-1	Day-7	Day-14	Day-21
I	Naïve	180.9 ± 4.96	173.8 ± 6.24	176.1 ± 5.82	177.4 ± 6.02
II	CFS	188.5 ± 5.33[a]	274.1 ± 4.50[a***]	269.8 ± 5.70[a***]	253.8 ± 6.17[a***]
III	CFS+CCE-125 mg/kg	181.2 ± 5.84[b]	193.6 ± 7.13[b***]	165.5 ± 8.53[b***]	158.2 ± 5.28[b***]
IV	CFS+CCE-250 mg/kg	179.6 ± 7.61[b]	174.5 ± 6.51[b***]	136.3 ± 5.06[b***]	131.6 ± 4.98[b***]
V	CFS+PG-100 mg/kg	185.4 ± 6.72[b]	186.9 ± 5.87[b***]	148.4 ± 6.22[b***]	142.6 ± 5.16[b***]

For comparison between four groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., n = 6. Normal group comprises vehicle treated animals who were not subjected to chronic forced swimming (CFS), control refers to vehicle treated group subjected to CFS, CEE refers to *C. cristata* extract and PG refers to *P. ginseng* extract. [a] as compared to naïve control and [b] when compared to chronic forced swimming control group of the same day. * represented $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

3.3. Locomotor activity

Aged rat showed restricted movements. CFS reduced movements in rats significantly within 21 days compared to naive control rats. CCE treatment significantly improved the spontaneous movement dose dependently with respect to CFS rats, similar to standard PG (Table 1).

3.4. Depression score—immobility periods

There was significant increase in the immobility period in CFS control group as compared to day-1. In control animals, CFS enhanced immobility period at day-21 compared to day-1. On the other hand, CCE treatment (125 and 250 mg/kg) for 21 days significantly decreased the immobility periods compared to the respective aged control. PG also showed anti-depressive action in comparison to CFS control (Table 2).

3.5. Anxiety score – elevated plus maze test

A state of anxiety in CFS control rats was shown by increase in time spent in the closed arm and less time spent in the open arm, and less number of entries in the open arm in the maze on observation of days-7, 14 and 21 when compared to day-1 (Tables 3 and 4). CCE treated group of rats spent significantly more time also in the open arm and decreased the time spent in the closed arm as compared to CFS control similar to prototype PG (Table 5).

3.6. Biochemical studies in brain region

Chronic forced swimming daily for 21 days accelerated oxidative stress as evidenced by significant enhancement in lipid peroxidation, nitrite and SOD levels, and a decrease in catalase level in whole brain of CFS groups compared to naïve control. Treatment with CEE (125 and 250 mg/kg) significantly reversed the CFS-induced oxidative stress as it decreased elevation of lipid peroxidation, nitrite and SOD level in brain as well as significantly increasing the catalase level. PG also showed effective results to combat oxidative stress resulted by CFS.

4. Discussion

The biggest challenge with geriatric impediment is that in most of the cases, the condition cannot be attributed to a single cause or in certain conditions of neuropsychiatric disorders like senile dementia or Alzheimer's depression [18,45,46]. There is also considerable evidence that stress-induced activation of the HPA (hypothalamic-pituitary-adrenal) axis causes loss of hippocampal spines, inhibition of hippocampal cell proliferation, and cognitive impairment [31,47,48]. Chronic fatigue syndrome (CFS) is differentiated by cognitive difficulties and exercise-induced fatigue as well as symptoms of immunologic dysfunction of all age groups [22,49,50]. Evidence of oxidative damage of DNA and lipids in tissues, especially in brain and muscles points to oxidative stress mechanisms in CFS [27–31]. In the present study, the orchid *C. cristata* Lindley was examined for its role in CFS induced behavioural changes in aged animals. Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, sterols in hydro-alcoholic extract of *C. cristata* (CCE). Further, chromatographic analysis identified and measured the phenanthrene analogue, coeloginanthridin, 9,10-dihydrophenanthrene in CCE (67.97%) that may be considered as an active biomarker [12,33]. Herbs from Orchidaceae family, containing naturally occurring phenanthrene compounds have been reportedly used for their cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, anti-platelet aggression, anti-allergic, immunomodulatory, anticancer and atherosclerosis properties [13–15,51]. In the present context, CCE did not show any signs and features of toxicity or mortality up to 2.0 g/kg per oral dose in Wistar rats. The results of the present study confirmed no observed adverse effect level (NOAEL) of CEE can be defined as more than 2 g/kg in Wistar rats and was identified as non-toxic. Further, oral treatment of CCE for three weeks significantly increased motor activity. Moreover, CCE enhanced

Table 4
Effect of *C. cristata* extract and *P. ginseng* extract on time spent in open arm in plus maze in chronic fatigue aged rats.

Groups	Treatments	Open arm time (sec)			
		Day-1	Day-7	Day-14	Day-21
I	Naïve	31.4 ± 2.85	27.2 ± 1.69	31.5 ± 1.58	29.8 ± 1.73
II	CFS	27.5 ± 2.99[a]	16.8 ± 1.91[a**]	18.6 ± 1.52[a**]	21.4 ± 1.05[a**]
III	CFS+CCE-125 mg/kg	34.6 ± 2.90[b]	40.9 ± 2.08[b***]	50.6 ± 2.37[b***]	52.6 ± 2.66[b***]
IV	CFS+CCE-250 mg/kg	36.3 ± 1.73[b]	53.1 ± 2.21[b***]	67.1 ± 2.62[b***]	71.5 ± 2.42[b***]
V	CFS+PG-100 mg/kg	29.2 ± 1.86[b]	44.7 ± 2.74[b***]	59.3 ± 2.86[b***]	64.8 ± 2.09[b***]

For comparison between four groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., n = 6. Normal group comprises vehicle treated animals who were not subjected to chronic forced swimming (CFS), control refers to vehicle treated group subjected to CFS, CEE refers to *C. cristata* extract and PG refers to *P. ginseng* extract. [a] as compared to naïve control and [b] when compared to chronic forced swimming control group of the same day. * represented $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 5
Effect of *C. cristata* extract and *P. ginseng* extract on number of entries in open arm in plus maze in chronic fatigue aged rats.

Groups	Treatments	Number of entries (5 min)			
		Day-1	Day-7	Day-14	Day-21
I	Naïve	4.7 ± 0.28	4.6 ± 0.32	5.1 ± 0.31	5.5 ± 0.46
II	CFS	5.0 ± 0.36[a]	3.8 ± 0.30[a**]	2.8 ± 0.32[a**]	2.6 ± 0.44[a**]
III	CFS+CCE-125 mg/kg	5.1 ± 0.47[b]	4.5 ± 0.34[b]	4.0 ± 0.26[b*]	4.3 ± 0.31[b*]
IV	CFS+CCE-250 mg/kg	5.2 ± 0.42[b]	5.1 ± 0.54[b]	4.6 ± 0.34[b*]	4.7 ± 0.28[b**]
V	CFS+PG-100 mg/kg	4.8 ± 0.39[b]	4.7 ± 0.38[b]	4.4 ± 0.31[b*]	4.4 ± 0.35[b*]

For comparison between four groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., n = 6. Normal group comprises vehicle treated animals who were not subjected to chronic forced swimming (CFS), control refers to vehicle treated group subjected to CFS, CEE refers to *C. cristata* extract and PG refers to *P. ginseng* extract. [a] as compared to naïve control and [b] when compared to chronic forced swimming control group of the same day. * represented $p < 0.05$ and ** $p < 0.01$.

the endurance of swimming activity or alternatively reduced the depression level in aged rats. Depression is one of the commonest psychological phenomenons in aging process. The present findings indicated that CCE has anti-depressive action and effective in attenuating CFS especially on aging animals, providing scientific evidence for the Ayurvedic practice in India.

Oxidative stress has long been linked to the neuronal cell death that is associated with certain neurodegenerative conditions, particularly during aging [48]. The present study confirmed the oxidative damage in rat brain due to the cause of CFS as evident by the elevation of lipid peroxides and nitrite and superoxide dismutase; while catalase was lowered [25,27,30]. Among the antioxidant enzymes, catalase is the first line of defence against oxidative injury. Actually, during oxidative stress with other harmful radicals hydrogen peroxides are also formed. Catalase has the ability to neutralize hydrogen peroxides in the tissues particularly in oxidative stress. Treatment with CCE reversed the situations like, *P. ginseng*. Previous studies suggested that *P. ginseng*, a well known traditional Chinese medicine is a longevity-promoting herb and helps the body to sustain better with stress and also optimizes the functioning of many bodily systems [32,52–54]. Our findings suggested that phenanthrenes are the active component of CCE and it might have potential therapeutic actions on CFS and related oxidative stress [13,35]. Of course, there are other mechanisms which operate whenever there is stress or anxiety, like ventral hippocampus as also other paradigms are there which are markers of HPA axis function like pituitary hormones or their downstream effects, but those were not dealt with this study [54,55]. So, their involvement in the anti-stress effect of CCE could not be ruled out.

5. Conclusion

These results indicate that the beneficial effect of CCE on chronic fatigue syndrome may possibly be due to its anti-anxiety, antidepressive and antioxidant properties. It is also hypothesized that phenanthrenes present on CCE may be responsible for its biological actions and one of the reasons may be through modulating antioxidant enzymatic pathways.

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Conflicts of interest

None.

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