Original Research Article (Experimental)

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

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**A B S T R A C T**

*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 pseudobulbs 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 improved (*p* < 0.001) the catalase level significantly 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.

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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, *coeloginanthrin* (3,5,7-trihydroxy-1,2-dimethoxy-9,10-dihydrophenanthrene) and *coeloginantrin* (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...
stilbene precursors and possess several biological activities [13]. Phenanthrenes have been studied for cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, anti-platelet aggregation, 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 glucocorticoid 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 30 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...
For comparison between three groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., *p < 0.05, **p < 0.01 and ***p < 0.001.

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 measuring 10 cm, with 40-cm high walls. The arms are connected by a central square, crossed with two opposite enclosed arms of the same dimension/C2.

For comparison between four groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., *p < 0.05, **p < 0.01 and ***p < 0.001.

Table 2

| Groups | Treatments     | Immobility score in 5 min |
|--------|----------------|---------------------------|
|        |                | Day-1                     | Day-7   | Day-14 | Day-21 |
| I      | Naive          | 108.5 ± 2.23              | 146.4 ± 3.18 | 178.9 ± 3.46 | 194.7 ± 4.19 |
| II     | CFS            | 102.6 ± 2.18              | 118.7 ± 2.09*** | 127.4 ± 2.72*** | 138.5 ± 2.93*** |
| III    | CFS + CCE-125 mg/kg | 108.6 ± 1.72              | 114.5 ± 2.84*** | 121.8 ± 2.95*** | 129.2 ± 3.14*** |
| IV     | CFS + CCE-250 mg/kg | 104.7 ± 1.95              | 120.1 ± 2.78**  | 131.7 ± 2.87**  | 142.6 ± 3.86**  |
| V      | CFS + PG-100 mg/kg | 104.7 ± 1.95              | 120.1 ± 2.78**  | 131.7 ± 2.87**  | 142.6 ± 3.86**  |

For comparison between three groups, multiple group comparison test was performed. Values were expressed as mean ± S.E.M., *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to chronic forced swimming control group of same day.
P. ginseng

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. * as compared to naive control and † 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 naive 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 (CEE). Further, chromatographic analysis identified and measured the phenanthrene analogue, coelegininanthridin, 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, spasmylytic, anti-inflammatory, anti-platelet aggregation, anti-allergic, immunomodulatory, anticancer and atherosclerosis properties [13–15,51]. In the present context, CEE 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, CEE enhanced

Table 3

| Groups | Treatments | Closed arm time (sec) | Day-1 | Day-7 | Day-14 | Day-21 |
|--------|------------|-----------------------|-------|-------|--------|--------|
| I      | Naive      | 180.9 ± 4.99          | 173.8 ± 6.24 | 176.1 ± 5.82 | 177.4 ± 6.02 |
| II     | CFS        | 189.5 ± 5.33          | 274.2 ± 4.50*** | 269.8 ± 5.70*** | 253.8 ± 6.17*** |
| III    | CFS + CCE-125 mg/kg | 191.6 ± 7.13*** | 203.6 ± 6.83*** | 195.6 ± 8.37*** | 195.6 ± 8.37*** |
| IV     | CFS + CCE-250 mg/kg | 179.6 ± 7.61b      | 174.3 ± 6.51*** | 136.3 ± 5.06*** | 131.6 ± 4.98*** |
| V      | CFS + PG-100 mg/kg | 185.4 ± 6.72b      | 168.9 ± 5.87*** | 148.4 ± 6.22*** | 142.6 ± 5.16*** |

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. * as compared to naive control and † when compared to chronic forced swimming control group of the same day. * represented p < 0.05, **p < 0.01 and ***p < 0.001.

Table 4

| Groups | Treatments | Open arm time (sec) | Day-1 | Day-7 | Day-14 | Day-21 |
|--------|------------|---------------------|-------|-------|--------|--------|
| I      | Naive      | 31.4 ± 2.85         | 27.2 ± 1.69 | 31.5 ± 1.53 | 29.8 ± 1.73 |
| II     | CFS        | 27.5 ± 2.99a        | 16.8 ± 1.91* | 18.6 ± 1.52*** | 21.4 ± 1.05*** |
| III    | CFS + CCE-125 mg/kg | 34.6 ± 2.08*** | 40.9 ± 2.08*** | 50.6 ± 2.37*** | 52.6 ± 2.66*** |
| IV     | CFS + CCE-250 mg/kg | 36.3 ± 1.73b      | 53.1 ± 2.21*** | 67.1 ± 2.62*** | 71.5 ± 2.47*** |
| V      | CFS + PG-100 mg/kg | 29.2 ± 1.86b      | 44.7 ± 2.74*** | 59.3 ± 2.86*** | 64.8 ± 2.09*** |

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. * as compared to naive control and † when compared to chronic forced swimming control group of the same day. * represented p < 0.05, **p < 0.01 and ***p < 0.001.
the endurance of swimming activity or alternatively reduced the depression level in aged rats. Depression is one of the most commonest psychological phenomena 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, anti-depressive 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 anti-oxidant enzymatic pathways.

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Conflicts of interest

None.

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References

[1] Joshi GC, Tewari LM, Lohani N, Upeti K, Jalal JS, Tewari G. Diversity of orchids in Uttarakhand and their conservation strategy with special reference to their medicinal importance. Rep Opin 2009;1:47–52.
[2] Gutierrez RMP. Orchids: a review of uses in traditional medicine, its phytocchemistry and pharmacology. J Med Plant Res 2010;4:392–638.
[3] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants, publications and information directorate. New Delhi, India: CSIR; 1965.
[4] Lele RD. Rejuvenation of the elderly. Bombay, India: Bhartiya Vidya Bhavan; 1986.
[5] Marani H, Pushpan R, Nishetwar K. Multi faceted actions of orchids in ethnomedicine—an appraisal. Int J Pharmaceut Biol Arch 1986;3:996–1002.
[6] Singh S, Singh AK, Kumar S, Kumar M, Pandey PK, Singh MCK. Medicinal properties and uses of orchids: a concise review. Elixir Apot Bot 2012;52:11627–34.
[7] Nag BC. Commentray on Caraka Samhita (Bengali). 1st ed. Hindawi Publishing Corporation; 1984.
[8] Singh DS. Trends in geriatric medicine. 1st ed. Varansi, India: Geriatric Society of India; Tara Printings Works; 1996.
[9] Datta HS, Mitra SK, Parmesh R, Patwardhan B. Theories and Management of Ageing: Modern and Ayurveda Perspectives. Evid Based Complement Alternat Med 2011. article ID 528527.
[10] Majumder PL, Banerjee S, Matti DC. Stilbenoids from the orchids Agrostemma githago and Coelogyne flaccida. Phytochem 1995;39:649–53.
[11] Majumder PL, Sen S, Majumder S. Phenanthrene derivatives from the orchid Coelogyne cristizus. Phytochem 2001;58:581–6.
[12] Majumder PL, Banerjee S, Pal S. Four new stilbenoids from the orchids Coelogyne ochracea and Coelogyne cristata. J Indian Chem Soc 2011;88:1293–304.
[13] Kovacs A, Vasai A, Hohmann J. Natural phenanthrenes and their biological activity. Phytochem 2008;69:1084–110.
[14] Cheng KS, Ko FN, Teng CM, Wu YC. Antilplatelet and vasorelaxing actions of some benzoquinolinoine and phenanthrene alkaloids. J Nat Prod 1996;59:531–4.
[15] Estelles R, Martin JL, Milian L, O’Connor JE, Losa MM, Nicolau MC, et al. Effect of two phenanthrene alkaloids on angiotensin II induced leukocyte endothelial cell interactions in vivo. Br J Pharmacol 2003;140:1057–67.
[16] Chakrabarty M, Dutta GK, Ghosh S, Debnath PK. Induction of antioxidative enzyme by the Ayurvedic herb Desmotrichum biobium BI. in mice. Indian J Exp Biol 2001;39:485–6.
[17] Dutta GK, Chakrabarty M, Ghosh S, Debnath PK. Hepatoprotective effect of Desmotrichum biobium BI. in mice with carbon tetrachloride-induced liver damage. Biomed Res 2002;13:81–4.
[18] Agudera C. HPA axis responsiveness to stress: implication of healthy aging. Exp Gerontol 2011;46:90–5.
[19] Sapolsky RM. Glucocorticoids, stress and their adverse neurological effects: relevance to aging. Exp Gerontol 1999;34:721–32.
[20] Ferrari E, Magri F. Role of neuroendocrine pathways of cognitive decline during aging. Age Res Rev 2008;7:225–33.
[21] Cho HJ, Skowera A, Cleare A, Wessely S. Chronic fatigue syndrome: an update focusing on phenomenology and pathophysiology. Curr Opin Psychiatry 2006;19:67–73.
[22] Fukuda K, Strauss SE, Hickie I, Sharpe MC, Dobbins JC, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Intern Med 1994;121:953–9.

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      | Naive      | 4.7 ± 0.28 | 4.6 ± 0.32 | 5.1 ± 0.31 | 5.5 ± 0.46 |
| II     | CFS        | 5.0 ± 0.36b | 3.8 ± 0.30*** | 2.8 ± 0.32*** | 2.6 ± 0.44*** |
| III    | CFS+CCE-125 mg/kg | 5.1 ± 0.47b | 4.5 ± 0.34b | 4.0 ± 0.26b | 4.3 ± 0.31b |
| IV     | CFS+CCE-250 mg/kg | 5.2 ± 0.42b | 5.1 ± 0.54b | 4.6 ± 0.34b | 4.7 ± 0.28*** |
| V      | CFS+FG-100 mg/kg | 4.8 ± 0.39b | 4.7 ± 0.38b | 4.4 ± 0.31b | 4.4 ± 0.35b |

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. * as compared to naive control and ** when compared to chronic forced swimming control group of the same day. * represented p < 0.05 and ** p < 0.01.
[23] Cleare AJ. The neuroendocrinology of chronic fatigue syndrome. Endocr Rev 2003;24:236–52.
[24] Chen R, Liang FX, Moriya J, Yamakawa H, Sumino H, Kanda T, et al. Chronic fatigue syndrome and the central nervous system. J Int Med Res 2008;36: 867–74.
[25] Logan AC, Wong C. Chronic fatigue syndrome: oxidative stress and dietary modification. Altern Med Rev 1986;6:450–5.
[26] Afari N, Buchwald D. Chronic fatigue syndrome: a review. Am J Psychiatry 2003;160:221–36.
[27] Lyle N, Bhattacharyya D, Sur TK, Munshi S, Paul S, Chatterjee S, et al. Stress modulating antioxidant effect of Nardostachys jatamansi. Indian J Biochem Biophys 2009;46:93–8.
[28] Lyle N, Gomes A, Sur TK, Munshi S, Paul S, Chatterjee S, et al. The role of antioxidant properties of Nardostachys jatamansi in alleviation of the symptoms of the chronic fatigue syndrome. Behav Brain Res 2009;202:285–90.
[29] Sur TK, Bhattacharyya D. The effect of Panax ginseng and diazepam on brain and hypothalamic 5-hydroxytryptamine during stress. Indian J Pharmacol 1997;29:318–21.
[30] Wang J, Sun C, Zheng Y, Pan H, Zhou Y, Fan Y. The effective mechanism of the polysaccharides from Panax ginseng on chronic fatigue syndrome. Arch Pharm Res 2013;36(2): 23963977.
[31] Mitra A, Dutta S, Mandal DN, Bhattacharyya K, Bhattacharyya D, Hazra J. Chemical characterization and antibacterial activity of Swarna jibanti (Coelogyn cristata Lindl.). Int J Ayu Pharm Chem 2015;3:299–315.
[32] OECD Guideline for the testing of chemicals. Acute oral toxicity in animals. OECD/OCDE No. 423, Adopted 21st September 1998.
[33] Lalremruba V, Prasanna SP. Evaluation of protective effect of Aegle marmelos Corr. in an animal model of chronic fatigue syndrome. Indian J Pharmacol 2012;44:351–6.
[34] Kulkarni SK, Kaur G. Chronic fatigue syndrome, pathophysiology and management. Express Pharma Pulse 1998;2:33–8.
[35] Kaur G, Kulkarni SK. Comparative study of antidepressants and herbal psychotropic drugs in a mouse model of chronic fatigue. J Chronic Fatigue Syndr 2000;6:23–34.
[36] Sapkota PS, Sur TK, Debnath PK, Bhattacharyya D. Effect of Peueria tuberosa on chronic foot shock stress in Wistar rats. Nepal Med Coll J 2010;12:234–8.
[37] Itoh J, Nabeshima T, Kameya T. Utility of an elevated plus maze for the evaluation of memory in mice: effects on nootropic, scopolamine and electroconvulsive shock. Psychopharmacol 1996;101:27–34.
[38] Byrde CM, Houghton LA, Pride CR. Effects of Aegle marmelos Corr. in an animal model of chronic fatigue syndrome. J Int Med Res 2008;36:551–6.
[39]不住み K, Kulkarni SK, Kaur G, et al. Chronic fatigue syndrome and the central nervous system. J Int Med Res 2008;36: 867–74.
[40] Logan AC, Wong C. Chronic fatigue syndrome: oxidative stress and dietary modification. Altern Med Rev 1986;6:450–5.
[41] Afari N, Buchwald D. Chronic fatigue syndrome: a review. Am J Psychiatry 2003;160:221–36.
[42] Lyle N, Bhattacharyya D, Sur TK, Munshi S, Paul S, Chatterjee S, et al. Stress modulating antioxidant effect of Nardostachys jatamansi. Indian J Biochem Biophys 2009;46:93–8.
[43] Lyle N, Gomes A, Sur TK, Munshi S, Paul S, Chatterjee S, et al. The role of antioxidant properties of Nardostachys jatamansi in alleviation of the symptoms of the chronic fatigue syndrome. Behav Brain Res 2009;202:285–90.
[44] Sur TK, Bhattacharyya D. The effect of Panax ginseng and diazepam on brain and hypothalamic 5-hydroxytryptamine during stress. Indian J Pharmacol 1997;29:318–21.
[45] Wang J, Sun C, Zheng Y, Pan H, Zhou Y, Fan Y. The effective mechanism of the polysaccharides from Panax ginseng on chronic fatigue syndrome. Arch Pharm Res 2013;36(2): 23963977.
[46] Mitra A, Dutta S, Mandal DN, Bhattacharyya K, Bhattacharyya D, Hazra J. Chemical characterization and antibacterial activity of Swarna jibanti (Coelogyn cristata Lindl.). Int J Ayu Pharm Chem 2015;3:299–315.
[47] OECD Guideline for the testing of chemicals. Acute oral toxicity in animals. OECD/OCDE No. 423, Adopted 21st September 1998.
[48] Lalremruba V, Prasanna SP. Evaluation of protective effect of Aegle marmelos Corr. in an animal model of chronic fatigue syndrome. Indian J Pharmacol 2012;44:351–6.
[49] Kulkarni SK, Kaur G. Chronic fatigue syndrome, pathophysiology and management. Express Pharma Pulse 1998;2:33–8.
[50] Kaur G, Kulkarni SK. Comparative study of antidepressants and herbal psychotropic drugs in a mouse model of chronic fatigue. J Chronic Fatigue Syndr 2000;6:23–34.
[51] Sapkota PS, Sur TK, Debnath PK, Bhattacharyya D. Effect of Peueria tuberosa on chronic foot shock stress in Wistar rats. Nepal Med Coll J 2010;12:234–8.
[52] Itoh J, Nabeshima T, Kameya T. Utility of an elevated plus maze for the evaluation of memory in mice: effects on nootropic, scopolamine and electroconvulsive shock. Psychopharmacol 1996;101:27–34.