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ORIGINAL ARTICLE

Evaluation of the Protective Effect of Ethanolic Extract of Seed Kernel of *Caesalpinia bonducella* Flem (EECB) on Forced Swimming-Induced Chronic Fatigue Syndrome in Mice

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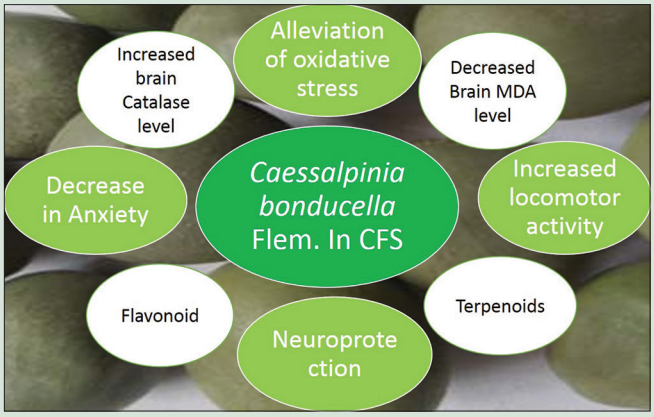
ABSTRACT

Objective: To study the protective effect of Ethanolic extract of seed kernal of *caessalpinia bonducella* Flem on forced swimming-induced chronic fatigue syndrome (CFS) in mice. **Materials and Methods:** Male albino mice of 25–40 g were grouped into five groups taking 5 mice in each group. Group A served as naïve control, Group B as stress control, and Group C and D received EECB at a dose of 200 mg/kg and 400 mg/kg, respectively. Group E was given the standard drug (imipramine 20 mg/kg). All animals received their respective agent orally daily for 7 days. Except for Group A animals, animals in all other groups were subjected to force swimming 6 min daily for 7 days to induce a state of chronic fatigue. Animals were assessed for duration of immobility on day 1, 3, 5, and 7. Level of anxiety (elevated plus maze and mirrored chamber test) and locomotor activity (open field test) were assessed 24 h after the last force swimming which was followed by estimation of oxidative biomarkers in brain homogenate. **Results:** Treatment with EECB (200 mg/kg and 400 mg/kg) and imipramine resulted in statistically significant ($P \leq 0.05$) reduction in anxiety and duration of immobility, and there was significant increase in locomotor activity when compared to stress control group. Significant reduction in malondialdehyde level and increase in catalase level were seen in EECB and imipramine-treated group compared to stress control group. **Conclusion:** The study confirms that EECB has protective effect against experimentally induced CFS.

Key words: *Caesalpinia bonducella* Flem, chronic fatigue syndrome, forced swimming, imipramine

SUMMARY

- Traditionally, the seed kernel of *Caesalpinia bonducella* Flem showed neuroprotection in many neurological conditions. In this study, we have evaluated the efficacy of ethanolic extract of *Caesalpinia bonducella* (EECB) in treatment of chronic fatigue syndrome. Treatment with chronic fatigue syndrome (CFS) resulted in decrease in duration of immobility, decreased anxiety, increased locomotor activity, decreased level of catalase, and increased level of malondialdehyde. These findings highlight the protective efficacy of EECB in treatment of CFS.



Abbreviations Used: CFS: Chronic fatigue syndrome, MDA: Malondialdehyde, EECB: Ethanolic extract of Seed Kernel of *Caesalpinia bonducella* Flem, TBA: Thiobarbituric acid, GABA: Gamma-aminobutyric acid, BH4: Tetrahydrobiopterin.

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INTRODUCTION

Chronic fatigue syndrome (CFS) is a heterogeneous disorder[1] characterized by persistent and unexplained fatigue, which results in severe impairment of daily functioning.[2] CFS patients complain of headache, gastrointestinal disturbance, paresthesia, cognitive dysfunction, neuropsychiatric problems (e.g., anxiety),[3] depression, and immunological disturbances.[1] Oxidative stress[4,5] and nitric oxide (NO) are being proposed in pathophysiology of CFS.[3] Psychological and physical stress may elicit the onset of CFS.[2] Even moderate psychological stress can induce cytokines and oxidative and nitrosative stress pathways.[6] Other precipitating events are mononucleosis, Lyme disease, and Q fever.[2]

The stressors increase level of NO in the body. NO through its potent oxidant product peroxynitrite, initiates the NO/ONOO- cycle, which is proposed to be the cause of CFS.[7] Elevated peroxynitrite level leads to mitochondrial dysfunction, hypothalamus pituitary adrenal dysfunction,[3] single-strand nicks on DNA, and depletion of NAD/NADH pools.[7] Furthermore, it causes decrease in natural killer cell function and other immune dysfunction.[7] Other aberrations observed are increased production of key inflammatory mediators – nuclear factor kappa B (NF-κB), cyclooxygenase 2, inducible NO synthase, increased pro-inflammatory cytokines, increased level of radical oxygen, autoimmune responses against oxidatively modified fatty acids and nitrated proteins, and lowered carnitine and coenzyme Q10 levels.[6] In

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mouse models of CFS, chronic fatigue was well correlated with markers of oxidative stress.[7,8]

Various anti-oxidants were found to be useful in treatment of CFS.[1,7] Imipramine and citalopram were found to have neuroprotective effect against CFS-induced biochemical and behavioral alterations.[3]

Caesalpinia bonducella Flem, belongs to the family *Fabaceae*, is a shrub which is widely distributed all over the world, especially in India and Sri Lanka.[9] The seed is also known as Latakaranja in Sanskrit, Kathkaranj in Hindi, and Fever nut in English. Various parts of the plants are reported to have antidiabetic, antiasthmatic, antioxidant, anti-inflammatory, antifilarial, antibacterial, immunomodulatory, antitumor, and anxiolytic activity.[10]

Many studies revealed that ethanolic extract of seed kernel of *C. bonducella* Flem (EECB) has high antioxidant activity.[11,12] Hence, this plant was selected for the study.

MATERIALS AND METHODS

Collection and authentication of plant material

Seeds of *C. bonducella* Flem were collected from local market in Dibrugarh and identified by Dr. L. R. Saikia, Professor, Department of Life Science, Dibrugarh University (Voucher specimen No DUL. Sc. 463/2013). A voucher specimen was deposited in the herbarium of the institute.

Preparation of plant extract

The seed kernels of *C. bonducella* Flem were manually separated from the outer shell, air-dried, and powdered. About 1100 g of powder was obtained which was then packed into Soxhlet apparatus, and extraction was done by continuous hot percolation using ethanol (95% v/v) as solvent. The extract was concentrated using rotary evaporator. It was further concentrated and dried in desiccators. The final yield of ethanolic extract was found to be 10.24% (w/w).

Phytochemical analysis

EECB was subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, sterols, terpenoids, and others as per standard methods.[13]

Drugs and chemicals

Imipramine was bought from Abbott Healthcare Pvt. Ltd., (Solan). Diazepam was procured from Ranbaxy Laboratories Limited (Solan). Ethanol was procured from Merck (Mumbai, Maharashtra, India). Hydrogen peroxide and tricarboxylic acid were procured from Sigma Aldrich Private Limited, Bangalore. Thiobarbituric acid (TBA) reagent was bought from Himedia Laboratories Private limited.

Experimental animals

Healthy male Swiss albino mice (25–40 g) were taken from the Central Animal House, Assam Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages under standard conditions of 12-h light and dark cycle and normal room temperature. Animals were fed with normal diet and water *ad libitum*. Before starting the study, permission from the institutional animal ethics committee was taken. The study was conducted according to CPCSEA guidelines.

Acute oral toxicity test

Acute oral toxicity test was done following OECD guidelines 425 (up and down method). EECB was found safe up to 2000 mg/kg dose.[14] Two arbitrary doses, i.e., 200 mg/kg and 400 mg/kg were selected for the study.

Experimental design

Animals were randomly assigned to five groups with five animals in each ($n = 5$).

1. Group A: Naïve animals (neither subjected to stress nor given any drug or extract)
2. Group B: Subjected to force swimming (to induce CFS) for 7 days (stress control)
3. Group C: Subjected to forced swimming + EECB (200 mg/kg) for 7 days
4. Group D: Subjected to forced swimming + EECB 400 mg/kg for 7 days
5. Group E: Subjected to forced swimming + standard drug (imipramine 20 mg/kg) for 7 days.

Imipramine (20 mg/kg) was taken as reference standard.[15] EECB and imipramine were administered orally 1 h before forced swimming.

Induction of chronic fatigue syndrome: Forced swimming

Forced swimming for 7 days is a well-validated animal model of CFS.[1] The animals were forced to swim individually in a glass jar measuring 25 cm × 12 cm × 25 cm, filled with water at room temperature (22°C ± 3°C). The depth of water was kept constant at 15 cm throughout the experiment. Generally, after an initial period of vigorous activity, the animals assume a typical immobile posture. During the 6-min forced swimming period, total duration of immobility was measured. The animals were judged immobile when they ceased struggling movement of their limbs to keep their head above water. The increase in immobility period induced by continued forced swimming is considered as a situation analogous to CFS.[3] The duration of forced swimming to induce CFS is taken as 6 min daily for 7 days. Immobility period was measured on day 1, 3, 5, and 7.[3,8]

Elevated plus maze test

This paradigm is suitable for assessing unconditioned anxiety state in rodents.[16] The elevated plus maze (EPM) apparatus consisted of two covered arms (16 cm × 5 cm × 12 cm) and two open arms (16 cm × 5 cm). The arms extended from a central platform (5 cm × 5 cm). The maze was kept at a height of 25 cm from the floor.[17] Each mouse was placed individually at the center of the EPM with their heads facing toward open arm.[15] During the 5-min test, parameters observed were: (a) latency to enter into open arm, (b) time spent in open arm,[17] and (c) number of entries into open arm (one entry was counted when there was all four paw entry into that arm).[15] Readings were taken after 24 h of last forced swimming.[3]

Mirror chamber test

The mirror chamber apparatus consists of a mirrored cube (30 cm × 30 cm × 30 cm) open on one side constructed of 5 pieces of mirror with one side mirrored and opposite side painted dark brown. The container box (40 cm × 40 cm × 40 cm) has opaque black walls and white floor. Placement of the mirrored cube into the container box forms a five-centimeter corridor which completely surrounds the mirrored chamber. A sixth mirror is placed on the wall of container box in such a way that it faces the single open side of the mirrored chamber.[18]

Each mouse was placed individually in a fixed corner outside the mirror chamber. During the 5-min test session, parameters noted were (a) latency to enter into mirror chamber, (b) number of entry into mirror chamber, (c) total time spent in the mirror chamber, and (d) average time per entry (time/entry) in mirror chamber.[18]

Assessment of locomotor activity: Open field test

The open field apparatus was made of plywood and measured 72 cm × 72 cm with 36 cm high walls. The floor was divided into sixteen 18 cm × 18 cm squares by drawing blue lines with a marker. A central square of 18 cm × 18 cm was drawn in the middle of the apparatus. Each mouse was placed into one of the four corners of the open field, facing the center. The mouse was then allowed to explore the apparatus for 5 min. The behaviors scored were (1) line crossing: numbers of line crossed with all four limbs and (2) rearing: number of times the mouse stood on its hind legs in the open field.[19]

Assessment of oxidative stress

24 h after the last forced swimming, experimental animals were sacrificed by decapitation and whole brain was removed. 10% (w/v) brain homogenate was prepared in 0.1 M phosphate buffer at pH 7.4. The post nuclear fraction was obtained by centrifugation of the homogenate at 1000 g for 15 min at 4°C, and it was used for catalase (CAT) assay. For malondialdehyde (MDA) assay, brain homogenate was centrifuged at 12000 g for 60 min at 4°C.[1]

Biochemical assessment of brain homogenate included the following

Catalase assay

The CAT activity assay was carried out by Beers and Sizer.[20] 2.5 ml of phosphate buffer (pH 7.8, 65 μM) was added to 0.1 ml of supernatant and incubated for 30 min at 25°C. After transferring to a cuvette, absorbance was measured at 240 nm by spectrophotometer. After that, 650 μl of hydrogen peroxide solution (7.5 mM) was added to initiate the reaction. Change of absorbance was measured for 3 min. Values were expressed as μmol of H_2O_2/min/mg of proteins.

Assessment of lipid peroxidation

Malondialdehyde (MDA) level was estimated as described by Satoh.[21] 75 mg of TBA was dissolved in 15% trichloroacetic acid (TCA). To this, 2.08 ml of 0.2 N HCl was added. The final volume was made up to 100 ml using 15% TCA. 3.0 ml of this reagent was then added to 0.75 ml of brain homogenate. The test tubes were then kept in a boiling water bath for 15 min. They were cooled and centrifuged for 10 min at 10,000 rpm. Absorbance of the supernatant was read against the blank at 535 nm. The results were expressed in nmol/mg of protein.

Statistical analysis

Statistical tests were performed using SPSS software (SPSS Statistics for Windows, Version 22.0., IBM Corp., Armonk, NY, USA). The statistical significance between groups was analyzed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. ANOVA followed by Bonferroni's tests was used between Group C and Group D. $P < 0.05$ were considered as statistically significant.

RESULTS

Acute toxicity study

Acute oral toxicity test was done following OECD guidelines 425 (up and down method). EECB was found safe up to 2000 mg/kg dose.[12]

Phytochemical analysis

Phytochemical analysis of seeds of *C. bonducella* has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids.

Effect of EECB on duration of immobility in forced swimming test

The results of forced swimming test are shown in Table 1. The duration of immobility was significantly increased on day 3, 5, and 7 in Group B (stress control) when compared to Group A ($P < 0.05$). EECB (both 200 and 400 mg/kg) showed significant ($P < 0.05$) reduction in immobility period when compared to stress control group (Group B). Similar reduction in duration of immobility was also observed in imipramine-treated animals. There was no statistically significant difference between animals treated with EECB 200 mg/kg and EECB 400 mg/kg.

Effect of treatment on the level of anxiety in elevated plus maze test

The results are shown in Table 2. There was significant decrease in the number of entry into open arm and total time spent in open arm in stress control group (Group B) when compared to naive group ($P < 0.05$). EECB and imipramine treatment significantly increased the number of entry and total time spent in open arm when compared to stress control group (Group B). No statistically significant difference was observed between animals treated with EECB 200 mg/kg and EECB 400 mg/kg with regard to both these parameters.

Latency to enter into open arm was increased in the stress control group when compared to naïve group ($P < 0.05$). EECB and imipramine-treated animals showed a significant ($P < 0.05$) decrease in latency period when compared to Group B (stress control group). There was no statistically significant difference between animals treated with EECB 200 mg/kg and EECB 400 mg/kg.

Effect of EECB on level of anxiety in animals tested in mirror chamber test

Results are shown in Table 3. Latency to enter mirrored chamber increased significantly ($P < 0.05$) in stress control group compared to Group A (naive). EECB and imipramine-treated animals showed significant ($P < 0.05$) decrease in latency to enter mirror chamber when compared to Group B (stress control group). There was no significant difference between EECB 200 mg/kg- and EECB 400 mg/kg-treated animals with regard to latency to enter mirror chamber.

However, there was significant decrease in the number of entry, total time spent in mirrored chamber, and average time per entry in Group B (stress control group) compared to Group A (naive). All these parameters were significantly increased ($P < 0.05$) in EECB and imipramine-treated animals when compared to Group B (stress control group). Significant difference was observed between EECB 200 mg/kg- and EECB 400 mg/kg-treated animals with regard to total time spent in mirror chamber.

Assessment of locomotor activity by open field test

Results are shown in Table 4. A significant decrease ($P < 0.05$) in total line cross and rearing was observed in Group B (stress control group) when compared to Group A (naïve). EECB and imipramine treatment significantly increased ($P < 0.05$) both these parameters of ambulatory activity when compared to Group B ($P < 0.05$). No significant difference was seen between EECB 200 mg/kg- and EECB 400 mg/kg-treated animals.

Biochemical estimation of effect of EECB on level of Catalase and malondialdehyde in mice brain

The results are shown in Table 5. Animals of stress control group recorded significant decrease ($P < 0.05$) in CAT levels when compared to naïve (Group A). Significant elevation of CAT level was seen in EECB and imipramine-treated animals when compared to Group B (stress control group).

Table 1: Effect of ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem on duration of immobility of chronically fatigued animals

Group	Treatment	Duration of immobility (s)			
		Day 1	Day 3	Day 5	Day 7
A	Naïve	147.2±0.04	155.3±6.95	154.7±3.21	151.4±13.02
B	Stress control	146.3±9.93	204.7±6.82[a]	208.3±6.7[a]	210.8±6.41[a]
C	EECB (200 mg/kg)	144.3±0.72	143.3±2.71[b]	141.3±2.82[b]	140.1±2.52[b]
D	EECB (400 mg/kg)	148.1±0.61	142.3±1.92[b]	140.9±0.96[b]	139.6±0.78[b]
E	Imipramine (20 mg/kg)	151.3±2.24	138.4±2.4[b]	134.7±0.31[b]	130.9±0.03[b]

All values are expressed in mean±SEM. Analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests. [a]$P<0.05$ when compared to naïve group, [b]$P<0.05$ when compared to stress control group, [c]$P<0.05$ when compared to Group C. SEM: Standard error of mean; ANOVA: Analysis of variance; EECB: Ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem

Table 2: Effect of ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem on the performance of chronically fatigued animals in elevated plus maze test

Group	Treatment	Number of entries to open arm	Time spent in open arm (s)	Latency to enter open arm (s)
A	Naïve	3.2±0.6	26.2±0.23	108.9±0.64
B	Stress control	0.8±0.4[a]	7.6±0.04[a]	242.6±1.62[a]
C	EECB (200 mg/kg)	2.2±0.4[b]	23.5±0.9[b]	100.5±0.94[b]
D	EECB (400 mg/kg)	2.6±0.46[b]	27.6±0.5[b]	98.6±1.21[b]
E	Imipramine (20 mg/kg)	3±0.8[b]	29.4±0.42[b]	94.8±2.16[b]

All values are expressed in mean±SEM. Analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests. [a]$P<0.05$ when compared to naïve group, [b]$P<0.05$ when compared to stress control group, [c]$P<0.05$ when compared to Group C. SEM: Standard error of mean; ANOVA: Analysis of variance; EECB: Ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem

Table 3: Effect of ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem on performance of chronically fatigue animals in mirror chamber test

Group	Treatment	Latency (s)	Number of entry	Spent in mirror chamber (s)	
				Total time	Average time
A	Naive	133.8±0.47	2.4±0.2	35.6±0.4	14.2±1.1
B	Stress control	200.2±1.2[a]	0.8±0.2[a]	6.2±0.21[a]	7.5±0.8[a]
C	EECB (200 mg/kg)	126.4±0.11[b]	2.4±0.4[b]	30.4±1.2[b]	12.9±0.1[b]
D	EECB (400 mg/kg)	120.6±0.2[b]	2.8±0.2[b]	40.2±0.7[b,c]	14.2±0.2[b]
E	Imipramine (20 mg/kg)	114.4±1.41[b]	3±0.41[b]	44.4±1.8[b]	14.8±1.2[b]

All values are expressed in mean±SEM. Analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests. [a]$P<0.05$ when compared to naïve group, [b]$P<0.05$ when compared to stress control group, [c]$P<0.05$ when compared to Group C. SEM: Standard error of mean; ANOVA: Analysis of variance; EECB: Ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem

Table 4: Effect of ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem on the locomotor activity of chronically fatigue animals in open field test

Group	Treatment	Total lines crossed	Rearing
A	Naïve	64.8±4.082	11.9±1.14
B	Stress control	22.1±0.35[a]	2.2±0.21[a]
C	EECB (200 mg/kg)	70.4±2.41[b]	8.8±0.03[b]
D	EECB (400 mg/kg)	75.12±0.8[b]	9.6±0.4[b]
E	Imipramine (20 mg/kg)	79.3±1.16[b]	9.8±0.4[b]

All values are expressed in mean±SEM. Analyzed by one-way ANOVA followed by Dunnett's multiple 0 comparison tests. [a]$P<0.05$ when compared to naïve group, [b]$P<0.05$ when compared to stress control group, [c]$P<0.05$ when compared to Group C. SEM: Standard error of mean; ANOVA: Analysis of variance; EECB: Ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem

Table 5: Effect of ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem on levels of catalase and malondialdehyde in brain tissue

Group	Treatment	Catalase (μmol/min/mg of proteins)	MDA (nmol/mg of proteins)
A	Naïve	2.4±0.341	0.198±0.012
B	Stress control	1.04±0.321[a]	0.961±0.043[a]
C	EECB (200 mg/kg)	2.712±0.263[b]	0.306±0.012[b]
D	EECB (400 mg/kg)	2.992±0.048[b]	0.214±0.028[b]
E	Imipramine (20 mg/kg)	3.032±0.143[b]	0.201±0.093[b]

All values are expressed in mean±SEM. Analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests. [a]$P<0.05$ when compared to naïve group, [b]$P<0.05$ when compared to stress control group, [c]$P<0.05$ when compared to Group C. SEM: Standard error of mean; ANOVA: Analysis of variance; MDA: Malondialdehyde; EECB: Ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem

Again MDA level was significantly high in Group B compared to Group A ($P < 0.05$). EECB- and imipramine-treated animals showed significant reduction in the level of MDA when compared to stress control group (Group B). There was no significant difference between EECB 200 mg/kg- and EECB 400 mg/kg-treated animals with regard to both these parameters (CAT and MDA).

DISCUSSION

CFS is a disorder of unknown etiology. It presents with somatic symptoms such as headache, joint pain, paresthesia, persistent and relapsing fatigue, and neuropsychiatric symptoms such as cognitive dysfunction, anxiety, and depression.[3] The chronic illness seems to be is largely due to NO/$ONOO^-$ cycle initiated by potent oxidant peroxynitrite.[7] Hypersecretion of pro-inflammatory cytokines is also seen in patients with CFS.[22] This hypersecretion may be due to stimulation of NF-κB which leads to elevated levels of interleukin 1 beta (IL-1β), IL-6, IL-8, tumor necrosis factor-alpha, and interferon-gamma.[7] Hypersecretion of pro-inflammatory cytokines during stress caused by hypofunctional neuroendocrine counter regulation may be a reason for stress-induced exacerbation of fatigue in patients with CFS.[22]

In the present study, continued swimming of 6 min daily for 7 days in the stress control group (Group B) resulted in significant changes in behavioral parameters such as increased immobility period which is indicative of depression and fatigue. An increase in level of anxiety was seen in EPM and mirror chamber test. Decreased locomotor activity was seen in open field test. In brain, homogenate decrease in CAT and increased lipid peroxidation markers (MDA) were seen, which indicates increased oxidative stress. Hence, we can definitely say that continuous forced swimming for 7 days produced CFS like condition in mice of stress control group. However, treatment with imipramine 20 mg/kg and EECB in the dose of 200 and 400 mg/kg significantly reversed these parameters and protected the animals from CFS.

In our present study, chronic forced swimming for 7 days significantly increased the duration of immobility and decreased locomotor activity in stress control group. This is a sign of increased fatigue in the animals. In CFS, there is easy fatigability. This may be due to mitochondrial energy metabolism dysfunction due to oxidation of cardiolipin molecules in the inner mitochondrial membrane by superoxide.[7] This leads to lowered complex I, III, and IV activity and subsequent lowered oxygen utilization in tissues. Again, ATP is depleted by peroxynitrite, superoxide, and NO.[7] EECB treatment protected the animals from this easy fatigability. The protective action of EECB seems to be due to the presence of flavonoids which scavenge peroxynitrite and superoxide radical. Flavonoids also help to restore tetrahydrobiopterin (BH4) levels in CFS patients. BH4 is important for synthesis of melatonin.[7] Melatonin synthesized in pineal gland is reported to have free radical scavenging property and it also stimulates endogenous antioxidant activity.[1]

Chronic swimming also increased anxiety behavior of the animals of stress control group in both EPM and mirror chamber test. CFS is also associated with neuropsychiatric problems like anxiety-like behavior.[3] EECB-treated animals showed decreased level of anxiety. This antianxiety activity of EECB may be attributed to the flavonoid content present in the extract as flavonoids are known to modulate gamma-aminobutyric acid A ($GABA_A$) receptors. Flavonoids can activate $GABA_A$ receptors even in the absence of GABA.[23] In intact animals, activation of this receptor is associated with antianxiety actions.[18]

Free radical generation is a part of normal respiration and other routine cell activities including microbial defense and also consequence of chemical and radiation injury.[24] CAT is a tetrameric ubiquitous heme protein.[15] It is present in peroxisomes, and it directs degradation of hydrogen peroxide to water and molecular oxygen.[24] Triterpenoids (lupeol) present in the seed kernel of *C. bonducella* Flem protect tissues from oxidative stress by increasing the transcriptional activity of NRF_2, which induces expression of important cytoprotective enzymes such as superoxide dismutase and CAT.[9]

MDA is one of the most frequently used indicators of lipid peroxidation.[25] Lipid peroxidation provides supply of free radicals which initiates peroxidation leading to breakdown of erythrocyte membranes and oxidation of proteins and DNA.[15] MDA is mutagenic and carcinogenic. It reacts with DNA to form adducts to deoxyguanosine and deoxyadenosine.[26,27] Evidence of oxidative damage of DNA and lipids in the vastus lateralis muscle points toward oxidative stress in CFS.[15] EECB-treated animals showed significantly increased level of CAT and decrease in the level of MDA when compared to stress control group. This may be attributed to antioxidant property of the extract.

Many *in vitro* and *in vivo* studies showed that EECB has potent antioxidant property.[11,12] The antioxidant property of *C. bonducella* Flem may be due to the presence of flavonoids[7] and terpenoids.[28] Flavonoids are chain-breaking antioxidants. They also scavenge peroxynitrite and superoxide. They also lowers NF-κB activity and helps to restore BH4 level.[5] Terpenoids also possess antioxidant property.[28] Ethanolic extract of *C. bonducella* seeds were are also reported to scavenge superoxide.[11]

CONCLUSION

The present study concludes that the ethanolic extract of seed kernel of *Caesalpinia bonducella* Flem Possess significant protective effect against CFS. Further study is required for identification and isolation of its active constituents and to confirm its exact mechanism of protection so that it can be better projected as a therapeutic agent for CFS.

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Conflicts of interest

There are no conflicts of interest.

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