

# A Study of the Protective Effect of *Triticum aestivum* L. in an Experimental Animal Model of Chronic Fatigue Syndrome

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## ABSTRACT

**Background:** Oxidative stress plays a major role in the pathogenesis of chronic fatigue syndrome (CFS). Keeping in view the proven antioxidant activity of *Triticum aestivum* L., this study has been undertaken to explore the potential therapeutic benefit of this plant in the treatment of CFS. **Objective:** To study the protective effect of the ethanolic extract of the leaves of *Triticum aestivum* (EETA) in an experimental mice model of CFS. **Materials and Methods:** Five groups of albino mice (20-25 g) were selected for the study, with five animals in each group. Group A served as the naïve control and Group B served as the stressed control. Groups C and D received EETA (100 mg/kg and 200 mg/kg b.w.). Group E received imipramine (20 mg/kg b.w.). Except for Group A, mice in each group were forced to swim 6 min each for 7 days to induce a state of chronic fatigue. Duration of immobility was measured on every alternate day. After 7 days, various behavioral tests (mirror chamber and elevated plus maze test for anxiety, open field test for locomotor activity) and biochemical estimations (malondialdehyde [MDA] and catalase activity) in mice brain were performed. **Results:** Forced swimming in the stressed group resulted in a significant increase in immobility period, decrease in locomotor activity and elevated anxiety level. The brain homogenate showed significantly increased MDA and decreased catalase levels. The extract-treated groups showed significantly ( $P < 0.05$ ) improved locomotor activity, decreased anxiety level, elevated catalase levels and reduction of MDA. **Conclusion:** The study confirms the protective effects of EETA in CFS.

**Key words:** Catalase, ethanolic extract, imipramine, malondialdehyde, *Triticum aestivum* L

## INTRODUCTION

The chronic fatigue syndrome (CFS) is a clinically defined condition characterized by severe disabling fatigue and a combination of symptoms like self-reported impairments in concentration, short-term memory, sleep disturbances and musculoskeletal pain.<sup>[1]</sup> Most of the patients of CFS are 30-40 years of age, with a female preponderance. The pathophysiology of CFS is still unclear. However, abnormal biological processes are present in many patients with subtle abnormalities of the central nervous system (CNS) and of neuroendocrine regulation.<sup>[2]</sup>

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CFS is also considered as an atypical manifestation of major depression. An emotional response to disabling fatigue, viral or immune changes or alterations in brain physiology and overlapping symptoms have been held responsible for high rates of depression in CFS patients.<sup>[3]</sup> A recent comprehensive review of neuroendocrine studies suggests an altered physiological response to stress as an important cause of CFS based on abnormalities in the hypothalamic–pituitary–adrenal (HPA) axis and serotonin pathways detected in CFS patients.<sup>[4,5]</sup> About one-third of patients showed hypocortisolism, which appears to originate from a CNS source rather than a primary adrenal site.<sup>[6]</sup> Oxidative stress is an important postulation for the pathogenesis of CFS. There is evidence of free radical-mediated oxidative damage to DNA and lipids in the vastus lateralis muscle of CFS patients and elevated levels of methemoglobin, which is a marker for oxidative stress, pointing to an oxidative stress mechanism in CFS.<sup>[2]</sup>

A wide range of pharmacological substances including anticholinergics, steroids, monoamine oxidase inhibitors, nonsteroidal anti-inflammatory drugs and antidepressants have been used to treat CFS. Because of the unclear etiology, diagnostic uncertainty and the resultant heterogeneity of the CFS population, there are no firmly established treatment recommendations for CFS.<sup>[7]</sup>

*Triticum aestivum* L. (Hindi name - gehun, kanak; Sanskrit name - godhuma) is called as wheat grass, belonging to the family Gramineae. It is the world's largest edible grain cereal - grass crop, usually 60-150 cm in height, but may be as short as 30 cm. The stem is tufted, erect or semi-erect, hollow with thin walls. Nodes are present in the stem. Leaves are long and narrow, having glabrous or hairy on one or both surfaces.<sup>[8]</sup> The therapeutic qualities have been attributed to its nutritional content, including chlorophyll, vitamins (A, C and E), bioflavonoids, iron, minerals (calcium and magnesium) and amino acids.<sup>[9]</sup> Scientific studies have revealed antioxidant, antiulcer and anti-arthritic activities of *Triticum aestivum* L. and it is shown to lower the transfusion requirements in Thalassaemia patients.<sup>[10]</sup>

Keeping in view the proven antioxidant activity of *Triticum aestivum* L. and the definitive role of oxidative stress in the pathogenesis of CFS, this study has been undertaken to explore the potential therapeutic benefit of this plant in the treatment of CFS.

## MATERIALS AND METHODS

### Experimental animals

Twenty-five adult albino mice weighing between 15 and 30 g were obtained from the Central Animal House, Assam Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animal room was adequately ventilated and kept at room temperature and relative humidity of  $26 \pm 2^\circ\text{C}$  and 40–70%, respectively, with 12-h natural light–dark cycles. The animals were maintained on standard animal diet and water was provided *ad libitum* during the entire period of the experiment. Permission from the Institutional Ethical Committee for the laboratory use of animals was duly obtained.

### Collection, identification and extraction of plant materials

Fresh leaves of *Triticum aestivum* L. were collected from a local firm in Dibrugarh district, Assam. The plant was identified by Dr. L. R. Saikia, Professor of Life Sciences, Dibrugarh University, Assam (No. DU/LS/2012) and authenticated from the Botanical Survey of India, Shillong. The cleaned materials were dried in shade and ground to a

fine powder with the help of a mixer grinder. The powder was then packed into a soxhlet apparatus and subjected to hot continuous percolation using ethanol (95% v/v) as a solvent. The extract was concentrated under a vacuum evaporator. The extract was collected and finally stored in air tight glass containers in a refrigerator at  $2-8^\circ\text{C}$  for use in the experiments. A final yield of 11.1% w/w with respect to the original air-dried powder was obtained.

### Drugs and chemicals

Solvent and chemicals of analytical grade were procured from a local supplier. Ethanol was obtained from the Central Medical Store of the state department AMCH. Potassium phosphate buffer, hydrogen peroxide and tricarboxylic acid were obtained from Sigma Aldrich Private Limited, Bangalore, India. Thiobarbituric acid was obtained from Hi Media Laboratories Private Limited, Mumbai, India.

### Phytochemical analysis

The Ethanolic extract of *Triticum aestivum* L. (EETA) was subjected to qualitative phytochemical analysis for flavonoids, alkaloids, saponins, tannins, terpenoids, sterols and others using standard procedures.<sup>[11]</sup>

### Acute toxicity study

An acute oral toxicity test for the EETA was carried out as per the OECD Guidelines 425.<sup>[12]</sup>

### Forced swimming test

Animals were forced to swim in a glass jar ( $25 \times 12 \times 25$  cm) containing water at room temperature ( $22 \pm 3^\circ\text{C}$ ) to produce a state of chronic fatigue. The water depth was adjusted to 5 cm and animals were forced to swim for a 6 min test session for 7 days. The duration of immobility period, characterized by cessation of struggling movements to keep the head above water, was recorded for each animal. The enhancement of immobility period induced by continued force swimming was considered a situation similar to CFS. The duration of immobility was measured on the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> days of the experiment.<sup>[13]</sup>

### Experimental protocol

Weight-matched animals were randomly divided into five groups of five animals each ( $n = 5$ ).

- Group A: Naïve animals, neither subjected to stress nor given any drug/extract
- Group B: Stressed control, subjected to forced swimming for 7 days but without any drug/extract
- Group C: Subjected to forced swimming + 100 mg/kg b.w. of EETA for 7 days
- Group D: Subjected to forced swimming + 200 mg/kg b.w. of EETA for 7 days

- Group E: Subjected to forced swimming + imipramine 20 mg/kg b.w. for 7 days.

EETA and imipramine were administered orally 1 h prior to exposure to the forced swimming test.

### Assessment of anxiety in elevated plus maze and mirror chamber

Elevated plus maze consisted of two open arms (16 × 5 cm) and two covered arms (16 × 5 × 12 cm) extending from a central platform, which was elevated to a height of 25 cm from the floor. The animals were placed individually at the center of the elevated plus maze with their heads facing toward an open arm. After 24 h of the last recorded forced swimming test of the animals, anxiety was assessed by recording the latency to enter the open arm, number of entries into the open arm and average time spent per entry in the open arm of the elevated plus maze.<sup>[14]</sup>

Anxiety was also assessed using a mirror chamber. The mirrored cube (30 × 30 × 30 cm) was constructed of five pieces of mirrored glass with mirrored surfaces facing the interior of the cube. The container box (40 × 40 × 30.5 cm) had a white floor and opaque black walls. Placement of the mirrored cube into the center of the container formed a 5 cm corridor completely surrounding the mirrored chamber. A sixth mirror was placed on the container wall positioned such that it faces the single open side of the mirrored chamber. Luminance in the corridor surrounding the mirrored chamber was 200 lux, whereas within the minor compartment the luminance was 100 lux. Mice were exposed to the chamber of mirrors and evaluated only once to avoid habituation problems. Mice were placed at a single, fixed starting point at the same corner of the corridor and allowed free movement around the corridor and into the chamber of mirrors. During the 5 min test session, the following parameters were noted: (1) latency to enter the mirror chamber, (2) number of entries in the mirror chamber and (3) average time spent per entry into the mirror chamber. Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber.<sup>[15]</sup>

### Assessment of locomotor/exploratory activity

Locomotor activity of the mice was assessed by an open field test, 24 h after the last forced swimming test. The open field apparatus measured 72 × 72 cm, with 36 cm walls. Sixteen squares (18 × 18 cm) were drawn on the floor. Mice were allowed to explore the apparatus for 5 min after

placing them randomly into one of the four corners of the open field, facing the center. To assess the locomotor and exploratory activity, the following behaviors were scored during the 5-min sessions:

- Line crossing: Number of lines crossed with all four limbs
- Rearing: Frequency with which the mice stood on their hind legs.

A high frequency/duration of these behaviors indicates high locomotor/exploratory behavior and low anxiety levels.<sup>[16]</sup>

## BIOCHEMICAL ASSESSMENT OF OXIDATIVE STRESS

Twenty-four hours after the last forced swimming, all the animals were sacrificed by cervical decapitation. The brain was removed and 10% w/v brain homogenate was prepared with 0.1 M phosphate buffer solution (pH 7.4). The postnuclear fraction obtained by centrifugation of the homogenate at 1000 r.p.m. for 20 min at 4°C was used for catalase assay. For malondialdehyde (MDA) assay, the homogenate was centrifuged at 10,000 r.p.m. for 20 min.<sup>[17]</sup> The biochemical estimate of the brain homogenate included:

### Catalase assay

Catalase was measured in the brain homogenate by continuous spectrophotometric rate determination by the Beers and Sizer method for antioxidant status. Phosphate buffer (2.5 mL, pH 7.8) was added to the supernatant and incubated at 25°C for 30 min. After transferring into the cuvette, the absorbance was measured at 240 nm spectrophotometrically. Hydrogen peroxide was added and change in absorbance was measured for 3 min. The values are expressed as  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2/\text{min}/\text{mg}$  wet tissue.<sup>[18]</sup>

### MDA estimation

The MDA level was measured by a colorimeter using thiobarbituric acid-reactive substance by the Thiobarbituric acid (TBA) method for antioxidant status. Seventy-five milligrams of thiobarbituric acid was dissolved in 15% Tricarboxylic acid (TCA); to this, 2.08 mL of 0.2 N HCl was added. The volume was made up to 100 mL using 15% TBA. Three milliliters of this reagent is added to 0.75 mL of the brain homogenate. The test tubes were kept in a boiling water bath for 15 min. They were cooled and centrifuged for 10 min at 10,000 rpm. The results were expressed in nmol/mg of wet tissue. The absorbance of the supernatant was read against the blank at 535 nm. The results were expressed in  $\mu\text{mol}/\text{mg}$ .<sup>[19]</sup>

## STATISTICAL ANALYSIS

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. ANOVA followed by Bonferroni's tests was applied for comparison between Group C and Group D.  $P < 0.05$  were considered as significant.

## RESULTS

### Acute toxicity test

There was no mortality and no sign or symptom of toxicity was reported among the animals up to 2000 mg/kg. Therefore, the LD50 was considered to be more than 2000 mg/kg body weight.

### Phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids and saponins in the extracts.

### Effect of extract treatment on duration of immobility on chronically fatigued animals

The results of the forced swimming tests are depicted in Table 1. On Day 1, no significant difference was observed among the experimental groups. But, on Days 3, 5 and 7, there was progressive and significant reduction of immobility period on forced swimming test in the extract-treated groups (Groups B and C) compared with the stress control group (Group B). The imipramine-treated group also showed significant reduction of immobility period on Days 3, 5 and 7. On the other hand, the stressed group showed a significant ( $P < 0.05$ ) increase in immobility period compared with the naïve group.

### Effect of extract on the level of anxiety tested in the elevated plus maze and mirror chamber

The results of elevated plus maze [Table 2] show that the latency to enter into the open arm is significantly increased and the average time spent in the open arm is significantly decreased in the stressed control group in comparison with the naïve group, indicating an increased level of anxiety in the stressed group. The extract-treated groups showed a significant increase in the average time spent in

the open arm and a decrease in the latency to enter the open arm ( $P < 0.05$ ) than the stressed group, suggesting a decreased level of anxiety. These effects were comparable to that of the imipramine-treated group.

In the mirror chamber test [Table 3], the latency to enter into the mirror chamber was significantly increased and the average time spent per entry was significantly decreased in the stressed group compared with the naïve group, which was indicative of an increased anxiety level in the stressed group. The extract-treated groups reversed these changes significantly ( $P < 0.05$ ) in a dose-dependent manner, which is suggestive of a reduction in the level of anxiety in the extract-treated groups.

### Assessment of locomotor activity of the experiment groups by the open field method

Total line crossing and rearing in the open field test [Table 4] were significantly decreased in the stressed group compared with the naïve group. Both the parameters significantly increased in the extract-treated groups ( $P < 0.05$ ) when compared with the stressed group. The findings are suggestive of increased locomotor activity in the extract-treated groups and the imipramine-treated groups.

### Biochemical estimation of catalase and MDA levels in mice brain

The results are depicted in Table 5. The stressed control group showed a significant decrease in the catalase and increase in the MDA levels compared with the naïve group, which is suggestive of oxidative stress. The extract-treated groups showed a significant increase in the catalase levels and decrease in the MDA level ( $P < 0.05$ ), which is suggestive of the antioxidant effect of the extract.

## DISCUSSION

The forced swimming test for 7 days produced a CFS-like condition in the stressed control group (Group B), as evidenced by a significant increase in the immobility period, which is indicative of physical exhaustion and depression. The stressed group also showed an increased anxiety-like state, as suggested by elevated plus maze

**Table 1: Effect of EETA on duration of immobility on chronically fatigued animals**

Groups	Treatment	Duration of immobility (in s)			
		Day 1	Day 3	Day 5	Day 7
A	Naive	151.0±13.59	157.4±5.08	160.3±13.12	156.6±4.64
B	Stressed group	154.4±8.09	202.9±16.81 <sup>a</sup>	207.1±16.71 <sup>a</sup>	205.6±16.4 <sup>a</sup>
C	EETA 100 mg/kg b.w.	148.4±6.33	145.3±11.18 <sup>b</sup>	142.6±8.69 <sup>b</sup>	131.4±8.14 <sup>b</sup>
D	EETA 200 mg/kg b.w.	146.1±6.07	142.7±11.88 <sup>b</sup>	133.1±6.44 <sup>b,c</sup>	123.7±6.57 <sup>b,c</sup>
E	Imipramine 20 mg/kg b.w.	147.4±5.65	136.9±10.87 <sup>b</sup>	128.0±7.97 <sup>b</sup>	119.9±7.91 <sup>b</sup>

Values are expressed as mean±SEM (n=5). <sup>a</sup> $P < 0.05$ , when compared with the naïve group; <sup>b</sup> $P < 0.05$ , when compared with the stressed group. <sup>c</sup> $P < 0.05$  when compared with Group C, EETA=Ethanollic extract of *Triticum aestivum* L.

**Table 2: Effect of extract treatment on the level of anxiety in elevated plus maze**

Group	Treatment	Average time spent in the open arm	Latency to enter into the open arm
A	Naïve	19±2.63	121.3±5.27
B	Stress control	10.20±1.59 <sup>a</sup>	138.4±5.00 <sup>a</sup>
C	EETA (100 mg/kg b.w.)	20.57±3.01 <sup>b</sup>	118.7±4.17 <sup>b</sup>
D	EETA (200 mg/kg b.w.)	26.71±3.50 <sup>b</sup>	114.27±2.57 <sup>b</sup>
E	Imipramine (20 mg/kg b.w.)	27.86±3.10 <sup>b</sup>	110.4±3.89 <sup>b</sup>

Values are expressed as mean±SEM (n=5). One-way ANOVA followed by Dunnett's multiple comparison test is done. <sup>a</sup>P<0.05, when compared with the naïve group; <sup>b</sup>P<0.05, when compared with the stressed group, EETA=Ethanol extract of *Triticum aestivum* L.

**Table 3: Effect of extract treatment on the level of anxiety tested in the mirror chamber**

Groups	Treatment	Latency to enter the mirror chamber (seconds)	Average time spent per entry (seconds)
A	Naïve	140.9±13.68	12.66±1.461
B	Stress control	201.7±13.4 <sup>a</sup>	6.714±0.606 <sup>a</sup>
C	EETA (100 mg/kg b.w.)	138.7±5.10 <sup>b</sup>	10.83±1.191 <sup>b</sup>
D	EETA (200 mg/kg b.w.)	122.7±4.96 <sup>b,c</sup>	13.21±0.342 <sup>b</sup>
E	Imipramine (20 mg/kg b.w.)	121.6±6.69 <sup>b</sup>	15.68±1.342 <sup>b</sup>

Values are expressed as mean±SEM (n=5). <sup>a</sup>P<0.05, when compared with the naïve group; <sup>b</sup>P<0.05, when compared with the stressed group. <sup>c</sup>P<0.05 when compared with Group C, EETA=Ethanol extract of *Triticum aestivum* L.

**Table 4: Assessment of locomotor activity by the open field method**

Groups	Treatment	Total line crossing	Rearing
A	Naïve	61.57±3.03	10±2.225
B	Stress control	26.29±1.79 <sup>a</sup>	3.714±0.565 <sup>a</sup>
C	EETA (100 mg/kg b.w.)	72.71±3.55 <sup>b</sup>	8.143±0.857 <sup>b</sup>
D	EETA (200 mg/kg b.w.)	75.29±4.72 <sup>b</sup>	10.57±0.428 <sup>b</sup>
E	Imipramine (20 mg/kg b.w.)	78.57±2.66 <sup>b</sup>	11.71±0.644 <sup>b</sup>

Values are expressed as mean±SEM (n=5). One-way ANOVA followed by Dunnett's multiple comparison test is done. <sup>a</sup>P<0.05 when compared with the naïve group; <sup>b</sup>P<0.05 when compared with the stressed group, EETA=Ethanol extract of *Triticum aestivum* L.

**Table 5: Effect of EETA on catalase and MDA levels in mice brain**

Groups	Treatment	Catalase (µmol/min/mg protein)	MDA (µmol/mg protein)
A	Naïve	2.071±0.192	0.256±0.015
A	Stressed control	1.2±0.278 <sup>a</sup>	0.622±0.019 <sup>a</sup>
A	EETA (100 mg/kg b.w.)	2.311±0.161 <sup>b</sup>	0.462±0.021 <sup>b</sup>
A	EETA (200 mg/kg b.w.)	2.806±0.080 <sup>b</sup>	0.313±0.029 <sup>b</sup>
A	Imipramine (20 mg/kg b.w.)	2.909±0.310 <sup>b</sup>	0.534±0.016 <sup>b</sup>

Values are expressed as mean±SEM (n=5). <sup>a</sup>P<0.05, when compared with the naïve group; <sup>b</sup>P<0.05, when compared with the stressed group, EETA=Ethanol extract of *Triticum aestivum* L; MDA=Malondialdehyde

and mirror chamber test, decrease in locomotor activity indicating chronic fatigue as suggested by the open field test and increased MDA and decreased catalase level in the brain homogenate that is suggestive of increased oxidative stress. Treatment with the extract (EETA) reversed all these changes significantly ( $P < 0.05$ ).

Increase in the immobility period and reduced locomotor activity in the open field test of the stressed control group is suggestive of increased fatigue, which is a prime component of CFS. This could be because of the abnormalities in carnitine homeostasis that are the main causes of muscle fatigue and have a significant role in the etiology of the chronic fatigue. Carnitine-dependent metabolic processes relate mainly to the fatigue-resistant type I fibers, which are more dependent on oxidative metabolism and provide skeletal muscle with the ability to gain and to use energy from the environment.<sup>[20]</sup> It is proposed that this disturbance in carnitine homeostasis may be due to a reduction in the carnitine palmitoyltransferase-I activity as a result of accumulation of omega-6 fatty acids.<sup>[21]</sup>

Exercise or stress-induced exacerbation of fatigue experienced by patients with CFS may result from hypersecretion of pro-inflammatory cytokines during stress caused by a hypofunctional neuroendocrine counter regulation.<sup>[17]</sup> Increased permeability of the blood-brain barrier and abnormality of the HPA axis is seen in the animal model of forced swimming test for inducing CFS.<sup>[2]</sup> This is in concordance with the fact that a subset of patients with CFS globally shows features of hypocortisolism, which appears to originate from a CNS source and hypofunction of the HPA axis.<sup>[22]</sup> The extract and imipramine decreased the immobility period significantly, which suggests that both the drugs may have a common corrective mechanism of action for correction of these underlying factors responsible for exacerbation of fatigue in the stressed animals.

The chronic forced swimming test also led to increased anxiety-like behavior, as suggested by the elevated maze plus test and the mirror chamber test. A high prevalence of generalized anxiety disorder, irritability, emotional lability and other neuropsychiatric and cognitive dysfunctions are seen in a subset of patients with CFS.<sup>[4]</sup> TCA like imipramine and SSRI like citalopram, which are used in the treatment of CFS, inhibit 5-HT and norepinephrine reuptake. Their anti-anxiety effects appear to be related to the capacity of serotonin to regulate the activity of brain structures such as amygdala and locus coeruleus that are thought to be involved in the genesis of anxiety.<sup>[23]</sup> The extract also showed a significant

decrease in the anxiety level of the stressed group, which is suggestive of its neuroprotective effect and may involve a similar mechanism of action like imipramine in alleviating anxiety.

Oxidative stress has been implicated as a major contributing factor in the pathogenesis of CFS. Oxidative stress leads to elevated peroxynitrite levels, which causes mitochondrial dysfunction and lipid peroxidation and leads to elevated cytokine levels by positive feedback. The cytokines, in turn, cause the formation of nitric oxide that combines with superoxide to form the potent oxidant peroxynitrite thus continuing the cycle. Peroxynitrite damages mitochondria, and this may help explain mitochondrial dysfunction in CFS.<sup>[24]</sup> This oxidative damage is more likely a contributor to the biochemical as well as behavioral changes in CFS. In our study also there is evidence of increased oxidative stress in the stressed group as denoted by a significant decrease in the catalase and increase in the MDA level compared with the naïve group. This also re-confirms the observations of the above study.

Oxidative stress occurs from the generation of reactive oxygen species (ROS), e.g. hydrogen peroxide and superoxide, which overcomes the scavenging abilities of antioxidants. It is characterized by lipid peroxidation, damage to the DNA and protein. Catalase acts as an enzymatic defense system against ROS, which catalyzes the decomposition of hydrogen peroxide ( $H_2O_2$ ) to water and oxygen and protects cells from oxidative damage.<sup>[25]</sup> Catalase activity varies greatly between tissues, with highest activities in the liver, kidney and erythrocyte, and lowest activity present in the connective tissues.<sup>[26]</sup> Lipid peroxidation is an indicator of oxidative stress in cells and tissues. Polyunsaturated fatty acid peroxides generate MDA upon decomposition, and measurement of MDA has been used as an indicator of lipid peroxidation.<sup>[27]</sup> Treatment with EETA showed a dose-dependent increase in catalase activity and decrease in the MDA level in a significant manner. From the results of the Catalase (CAT) and MDA estimations, it may be stated that the ingredients present in the EETA possess antioxidative properties in an exhaustive swimming-induced oxidative stress condition. The major clinical utility of *Triticum aestivum* L. is due to its antioxidant action, which is derived from its high content of bioflavonoids like apigenin, quercetin and luteolin.<sup>[9]</sup> Quercetin has the ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation.<sup>[28]</sup> Apigenin is also shown to possess significant anti-inflammatory activity that involves blocking NO-mediated COX-2 expression and monocyte adherence.<sup>[29]</sup> This may probably attenuate the hypersecretion of pro-inflammatory cytokines seen in CFS patients that results in exacerbation of fatigue. The

protective effect of imipramine against oxidative stress in this study can be explained by the fact that one of the shared mechanisms of action of antidepressants is the up-regulation of antioxidant enzymes.<sup>[13]</sup>

Thus, considering all these results, it can be concluded that EETA could be beneficial for the protection of exhaustive physical exercise like swimming-induced oxidative stress injury on brain tissues and also on the vital tissues like cardiac, skeletal and hepatic tissues, which are very much prone to physical exercise-induced oxidative stress. Its neuroprotective effect on the behavioral alterations induced by the forced swimming test may involve common mechanisms with TCA like imipramine that may include correction of HPA abnormality, serotonin neurotransmission and modulation of nitric oxide pathway. But, further studies are needed to evaluate its precise mechanism of action so that it can be better projected as a good therapeutic agent for the benefit of mankind.

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