INTRODUCTION

Stress is an inevitable part of life, the relationship between stress and cognition is complex, and cognitive impairment due to stress depends on the type, duration, and severity. Stress is increasingly being recognized as the precipitant of several psychiatric illnesses including anxiety and depression [1]. Chronic variable stress models have been proven to be more useful as they are devoid of the problem of resistance in the animal species toward the commonly used stressors and also have the advantage of the development of effective and long-term stress response. Thus, chronic unpredictable stress (CUS) models are nowadays the preferred models for the generation of a stress response [2]. Chronic variable or unpredictable stress has shown to be consistent with the constellation of symptoms associated with post-traumatic stress syndrome, such as re-experiencing, and arousal to fearful contexts, validating CUS as a model for post-traumatic stress. Thus, chronic unpredictable stress disorder (PTSD) results from traumatic stress exposure. Cognition and mood symptoms can begin or worsen after the traumatic event. In this study, we aimed to evaluate the role of *Eclipta alba* Linn. as an antistressor, AChE inhibitor, and on object recognition memory in an animal model of PTSD.

METHODS

**Objective:** Acetylcholinesterase (AChE) inhibitors are important therapeutic targets to treat memory impairment caused due to stress, post-traumatic stress disorder (PTSD) results from traumatic stress exposure. Cognition and mood symptoms can begin or worsen after the traumatic event. In this study, we aimed to evaluate the role of *Eclipta alba* Linn. as an antistressor, AChE inhibitor, and on object recognition memory in an animal model of PTSD.

**Methods:** Adult male Wistar albino rats were randomly divided into four groups: Control, chronic unpredictable stress (CUS) (30 days), CUS+ethanolic extract of *E. alba* (EEEA) (200 mg/kg body weight), and EEEA (200 mg/kg body weight) treatment groups and were assessed for novel object recognition task (NORT), plasma corticosterone, and AChE activity.

**Results:** We found significant improvement in NORT (p<0.05) plasma corticosterone levels in stress group was significantly increased (p<0.00.05) which is resumed with EEEA treatment (p<0.005) AChE activity was found to be reduced after EEEA treatment.

**Conclusion:** EEEA is found to possess cognitive enhancing activity in the animal model of PTSD.

**Keywords:** Recognition memory, PTSD, Unpredictable stress, *Eclipta alba*
of six rats each: Group I served as control group; Group II animals were induced with CUS (Table 1); Group III animals were pretreated for 15 days with 200 mg/kg bw EEEA followed by 30 days CUS+EEEA treatment; IV group served as treatment group where animals were treated with 200 mg/kg body weight EEEA for 45 days.

**Induction of stress [22] (Modified Katz et al., 1982)**

Single stressor per day was given in an unpredictable manner with no single stressor repeating on consecutive days (Table 1).

**Drug treatment**

The dried whole plant of *E. alba* Linn was collected from Amruth Kesari Depot, Bangalore-S3. Identification and authentication was done by Dr. Aravind, Assistant Professor, Department of Medical Botany, National Institute of Siddha (Reg. No of the certificate: NIS/MB/92/2013). The whole plant was coarsely powdered and the extraction was done in succession using Soxhlet extraction method. All the extracts were made solvent free and concentrated using rotary evaporator and preserved at 4°C in the airtight bottle until further use.

**Dosage and route of administration**

EEEA was dissolved in warm normal saline (0.9% NaCl) dose-200 mg/kg body weight [23] was administered through the oral route. Drug was administered 1 hr before induction of stress in case of stress+group treatment.

**Plasma corticosterone assay**

Assay of corticosterone [24] is based on the oxidation of corticosteroids with ferric iron (III) in acidic medium and subsequent complex with ferrous iron (II) and potassium hexacyanoferrate. 0.5 µl was mixed with appropriate volumes of the working solutions of corticosterone and was transferred into a series of 10 ml volumetric askes. 2 ml of sulfuric acid and 2 ml of ferric chloride were added to 0.5 ml of potassium hexacyanoferrate (III) solution. This mixture was heated in a water bath maintained at 70°C±2°C for 30 minutes with occasional shaking and diluted to the 5 ml mark with distilled water. Absorbance was measured at 780 nm against the reagent blank.

**Assay of AChE [24]**

AChE activity was measured by modified Ellman’s method (Ellman et al., 1961). At the end of experimentation, rats were decapitated; the frontal cortex, hippocampus, and striatum were dissected quickly. The tissues were homogenized in 0.1 M phosphate buffer, pH 8.0. The reaction mixture consisted of 2.6 ml of phosphate buffer (0.1 M, pH 8.0), 0.4 ml aliquot of homogenate, and 0.1 ml of 0.01 M dithiobis nitrobenzoic acid. After addition of the substrate, acetylthiocholine iodide (0.075 M), the change in absorbance was noted every 2 minutes until the absorbance reached a constant value. The activity was expressed as micromoles hydrolyzed per minute per gram of tissue.

**NOR task (NORT) [25]**

NORT is a model of recognition memory and is based on the innate behavior of animals to spend more time in exploring new objects; the choice to explore new objects implicates learning of new information. The neuroanatomical substrate for this test is prefrontal cortex and hippocampus.

Open field arena (100×100×40) illuminated with 40 lux dim light was used for the test.

NORT was carried out in three phases.

1. **Habituation** – animals for habituated to the arena in two sessions of 10 minutes each with intersession interval of 4 hrs.
2. **Familiarization** – this session was conducted 24 hrs after habituation session. Two identical objects were placed 90 cm apart, time spent by each rat in exploring the objects was recorded for 5 minutes.
3. **Test** – the test session was conducted with an intra-trial interval of 1 hr. Each rat was presented with a familiar object placed at the same position and an novel object at the place of the second familiar object. The time spent exploring the familiar and novel object was recorded for 5 minutes.

Following were assessed by NORT:

1. The absolute time of novel object exploration (time in seconds)
2. An exploratory preference score, i.e., time spent exploring the novel object divided by the total time spent exploring both objects ×100. An exploratory preference score of 50% indicated chance performance, while higher preference scores indicate intact memory performance.
3. Discrimination index (exploration of novel object [sec]-familiar object [sec])/total time (sec).

**Statistical analysis**

The data were analyzed by one-way analysis of variance followed by Tukey’s multiple comparisons post-hoc test. Values are expressed as mean±standard deviation; p<0.05 was considered statistically significant.

**RESULTS**

**Plasma corticosterone (Fig. 1)**

Fig. 1 indicates the Effect of chronic unpredictable stress (CUS) and Ethanolic extract of *Eclipta alba* (EEEA) treatment on plasma corticosterone levels. Animals Exposed to 30 days of unpredictable stress showed increase in the levels of plasma corticosterone as compared with the control animals (p<0.05), when CUS animals were treated with 200 mg/kg bw of EEEA, significant decrease in the levels of plasma corticosterone was observed indicating restoration of HPA axis (p<0.05) and decrease in stress response. Treated group did not show any statistical changes when compared to Stress group animals (p<0.05).

| Day  | Stressor                                           | Duration |
|------|----------------------------------------------------|----------|
| 1, 7, 13, 19, 25 | Noise stress (100 dB, white noise generator) | 4 hrs |
| 2, 8, 14, 20, 26 | Cage isolation (small housing cage for rodents) | 24 hrs |
| 3, 9, 15, 21, 27 | Forced swim stress at 23°C±1°C                   | 15 minutes |
| 4, 10, 16, 22, 28 | Immobilization stress                            | 4 hrs |
| 5, 11, 17, 23, 29 | Food deprivation stress                          | 24 hrs |
| 6, 12, 18, 24, 30 | Overnight illuminated lights                      | 12 hrs |

**Table 1: Chronic unpredictable stress Protocol**

![Plasma Corticosterone](image)
Acetylcholinesterase activity (Figs. 2-4)
Fig 2-4 Acetyl cholinesterase activity was increased invariably in the Prefrontal cortex (Fig 2), Hippocampus (Fig 3), Corpus striatum (Fig 4) of Stressed animals (p<0.05), whereas significantly decreased in CUS exposed animals treated with 200 mg/kg bw EEEA (p<0.05). Treated alone group did not show any difference. *Compared to control and #Compared to CUS.

NORT (Figs. 5-7)
Exploration of Novel object is an innate behaviour of rats, when we subjected all four group rats to the NOR Task, we observed difference in the exploration behaviour.

Animals exposed to CUS spent considerably less time exploring the novel object (p<0.05) when compared with control and other groups.

Stressed rats showed less preference for Novel object (Fig 6) (p<0.05) as compared with control and other groups and upon treatment there was significant change, Preference towards novel object was significantly more (p<0.05).

Discrimination Index (DI) (fig 7) allows discrimination between the novel and familiar objects, it is the difference in exploration time for familiar object, dividing this value by the total amount of exploration of the novel and familiar objects [DI = (TN - TF)/(TN + TF)]. This result can vary between +1 and -1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score indicates a null preference. Our observation shows Negative score in case of Stressed group of animals (p<0.05), whereas positive score of other three groups shows more time spent with novel object as compared to stress group.

DISCUSSION
In the present study, male Wistar albino rats were chosen to mimic PTSD by inducing CUS. When animals exposed to different stressors...
related to cholinergic neuromodulation; ACh has been demonstrated to enhance the persistent spiking of individual cortical neurons, which could provide a mechanism for active maintenance of novel information. This effect has been shown in entorhinal cortex and could also enhance encoding by enhancing long-term potentiation. ACh enhances LTP in many areas, including the hippocampus [29-31]. Principal role of AChE is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of ACh. Inhibition of AChE serves as a strategy for the treatment of memory disorders [32]. Inhibitors of AChE and butyrylcholinesterase, key enzymes involved in the degradation of neurotransmitter ACh, have been shown to function by restoring the level of ACh in the synaptic region and thus reinstating deficient cholinergic neurotransmission [33,34]. Since the discovery of cholinergic deficits in patients suffering from neurological disorders, inhibition of these enzymes is the main target in the treatment strategies [32]. Synthetic drugs used in the treatment of cognitive dysfunction associated with Alzheimer’s disease and other diseases include tacrine, donepezil, and rivastigmine [35]. However, these drugs are associated with adverse effects including gastrointestinal disturbances, hepatotoxicity, and bioavailability problems [36-38]. E. alba, a dietary herb with potential anti-AChE activity [21], showed significant decrease in AChE in Frontal cortex, hippocampus, and striatum in CUS animals, which is also supported by an study where E. alba has shown to possess memory enhancing activity in scopolamine-induced amnesia model as assessed by in elevated plus maze model indicating its cognitive enhancement property [23].

In vitro study reveals that the neuroprotective effect of another herb Triphala has also found to be due to antioxidant and AChE inhibitory property [39]. Another study where four garden varieties of black tea indicated that both infusion and decoction of these tea varieties showed AChE inhibitory properties in a dose-dependent manner in mice [40].

Plants exhibiting anti-AChE activity are largely targeted for treating neuronal complications.

Our study also reveals the role of E. alba as AChE inhibitor thereby improving recognition memory in the animal model of PTSD. Findings of our study conclude that the EEEA, with rich phytochemical constituents, can emerge as a therapeutic agent useful in treating neuronal complications.
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