IMPAIRED OBJECT RECOGNITION MEMORY AND ACETYLCHOLINESTERASE ACTIVITY IN ANIMAL MODEL OF POST-TRAUMATIC STRESS DISORDER-RESTORED BY ECLIPTA ALBA LINN. A DIETARY HERB.

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Received: 19 July 2016, Revised and Accepted: 30 August 2016

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 disorder (PTSD) in animals [3]. Different types of stressors such as noise stress, psychological stressors have proven to alter neurochemical, neurobehavioral as well as neurotransmitter levels in different brain regions [4-6] and also individuals with PTSD shown to have smaller hippocampal volumes [7-9] and exhibit impaired performance on hippocampal-dependent tasks [10,11]. Stress affects long-term potentiation and epigenetically regulates the hypothalamic-pituitary-adrenal axis [12]. Recognition memory refers to the ability to judge a previously encountered item as familiar and depends on the integrity of the medial temporal lobe [13]. Investigations of the neural basis of recognition memory have implicated several brain regions. Recognition memory involving judgment of prior occurrence for individual items, relies on the perirhinal cortex, whereas recognition memory that involves multiple items and their contextual associations or the temporal order, in which items are encountered depends on interactions between the perirhinal cortex, hippocampus, and medial prefrontal cortex [14]. Recognition memory is strongly correlated with hippocampal and cortical functional integrity in both rodents’ and primates’ brains [15]. Moreover, both cortical and hippocampal dysfunctions affect novel object recognition (NOR) paradigm results [16]. Acetylcholine (ACh) is a neurotransmitter involved in memory and learning processing in the cholinergic system. Acetylcholinesterase (AChE) is the enzyme that decreases ACh levels by hydrolysis. The high AChE activity is present in the chronically stressed brain [6]. Eclipta alba Linnaeus. Family-Compositae commonly called as Bhringraj grows widely as an annual weed in moist places and is widely used in traditional system of medicine and is reported to possess antihyperglycemic, anesthetic, antioxidant, anti-inflammatory, antiaggressive, anti-fungal activities, and also a good hair growth regulator [17]. Phytochemically, E. alba is rich in wederolactone, β-amin, stigmastanol, and luteolin-7-glucoside [18,19]. Previous investigations have revealed that E. alba herbal supplementation can significantly control cognitive and motor neuron dysfunction in old age. Similarly, medicinal plants and their bioactive constituents can improve behavioral (motor and cognitive behavior), neuronal signaling, and anti-inflammatory effects [20]. Different extracts and its fractions of E. alba were analyzed for their antioxidant, AChE inhibitory, and antifungal activity, which have shown the best antioxidant capacities also have the most potential inhibitors of AChE [21]; hence, the present study was undertaken to evaluate the effect of herbal extract of E. alba (EEEA) on AChE activity and object recognition memory in animal model of PTSD.

METHODS

Experimental animals

Male Wistar rats, weighing 200-225 g (2-2.5 months old) obtained from the Central Animal House Facility from Dr. ALM PG Institute of Basic Medical Sciences, were used for the experiments. The rats were housed and maintained in standard laboratory conditions. Food and water were provided ad-libitum unless specified otherwise in stress protocols. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC No-01/25/2014). All efforts were made to minimize both the suffering and the number of animals used. Rats were randomly divided into 4 groups consisting...
of six rats each: Group I served as normal control; 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-53). 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+treatment group.

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 ml 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 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 was added in 0.01 M sodium molybdate buffer pH 8.0. The mixture was incubated at 37°C±0.5°C for 30 minutes with occasional shaking. The reaction was terminated with 2 ml of 20% trichloroacetic acid and 2 ml of ferric chloride. The absorbance was measured at 412 nm using an LKB spectrophotometer. The activity was expressed as micromoles of acetylcholine 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 200mg/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).

![Plasma Corticosterone](image)

Fig. 1: Effect of chronic unpredictable stress (CUS) and Ethanolic extract of Eclipta alba (EEEA) treatment on plasma corticosterone levels. All values expressed as mean±standard deviation. CUS exposed animals showed significantly increased plasma corticosterone as compared with control (p<0.05), upon treatment of EEEA, corticosterone levels were significantly reduced (p<0.05). *Compared to control and #Compared to CUS
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Fig. 2: Effect of chronic unpredictable stress (CUS) and ethanolic extract of *Eclipta alba* (EEEA) treatment on acetylcholinesterase (AChE) activity in prefrontal cortex. All values expressed as mean±standard deviation. AChE activity was significantly increased in animals exposed to 30 days of chronic unpredictable stress (p<0.05), whereas significantly decreased in CUS exposed animals treated with 200 mg/kg bw EEEA (p<0.05). *Compared to control and #Compared to CUS.

Fig. 3: Effect of chronic unpredictable stress (CUS) and ethanolic extract of *Eclipta alba* (EEEA) treatment on acetylcholinesterase (AChE) activity in hippocampus. All values expressed as mean±standard deviation. AChE activity was significantly increased in animals exposed to 30 days of chronic unpredictable stress (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.

ACHe 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 indicating degradation of Acetylcholine at these regions whereas same is restored to near control values upon treatment with EEEA 200mg/kg bw (p<0.05) indicating effectiveness of eclipta alba as Acetyl cholinesterase inhibitor; treatment group (EEEA alone) showed significantly lower AchE activity in the Hippocampus as compared to control (p<0.05) indicating memory enhancing activity even in the normal control animals upon EEEA treatment. whereas there was no change in Corpus striatum and frontal cortex between control and treated group.

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 \[DI = (\overline{TN} - \overline{TF})/(\overline{TN} + \overline{TF})\]. 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...
study reveals that the neuroprotective effect of another herb treatment in albino rats. J Pharmacol Sci as AChE inhibitor thereby and impairment in NORT [27,28]. This vital cognitive function has been social instability combined with predator stress exposure showed PTSD, i.e., 1 week exposure to single psychosocial stress and chronic in corticosterone levels observed in our study. when animals exposed to male Wistar albino rats. Hippocampus, the neural component was observed along with the memory deficit when stress was applied serum corticosterone level as well as hippocampal corticosterone changes following the stress in rat’s hippocampus [26]. Increase in levels might have implications for brain plasticity and behavioral types of stressors affecting memory through increased glucocorticoid abnormalities seen in chronic stress models of PTSD are due to the neurotoxic effects of corticosteroids, as recognition memory is a component of hippocampal function. In our study, a significant increase in plasma corticosterone levels in animals exposed to CUS, in accordance several studies have reported the relationship between the different and butyrylcholinesterase, key enzymes involved in the degradation of neurotransmitter ACh can be, as shown to be function 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 components. AChE inhibitory properties in a dose-dependent manner in mice [40].

REFERENCES


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 reinstate 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].

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