EFFECT OF ECLIPTA ALBA ADMINISTRATION ON CHRONIC STRESS INDUCED BEHAVIORAL CHANGES IN WISTAR ALBINO RATS

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ABSTRACT
Introduction: Stress is an unavoidable part of modern life. Chronic exposure of stress is triggers the cognitive decline. In this study, we assessed the effect of eclipta alba on acute and chronic unpredictable stress in retention of spatial memory, using a T-maze task. Methods: Adult albino rats were randomly divided into six groups. Rats exposed to acute and chronic unpredictable stress and treated with eclipta alba were compared with control animals and assessed for T-maze. Results: Our data shown that eclipta alba treated rats shown the significance (P<0.05) in decreased latency, number of days taken and increase in the correct choice percentage, when compared to the acute and chronic stress groups. Conclusion: The results revealed that eclipta alba is serving as a memory enhancer. It will be a better choice to be an antidote for stress induced memory impairment.

KEYWORDS: Acute and chronic Unpredictable stress, Eclipta alba, Spatial Memory, T-maze.

INTRODUCTION
Stress, which is becoming more prevalent in daily human life, has dual impacts on brain and body physiology. Stress, defined as a disturbance in homeostasis, was formulated by Hans Selye in his ‘General Adaptation Syndrome’ concept which described a biological stress syndrome produced by diverse noxious agents (Hans Selye, 1950). It is a state of threatened homeostasis that produces a different physiological as well as pathological changes depending on severity, type and duration of stress (Kumar et al., 2011). Stress as a functional response is necessary for successful coping with environmental challenges. It enables an organism to deal with the wide variety of aversive and undesirable situations present in everyday life facilitating survival in a continuously changing environment (Olff and Ursin, 1993).

Within limits, acute stress has positive effects, such as improved memory performance (De Kloet et al., 1999) and increased alertness, focus, and energy; furthermore, it can help people to cope with unfavorable situations. However, chronic stress takes a toll on physiology, as well as on mood, affecting productivity and quality of life and ultimately leading to affective disorders, such as anxiety, depression and other related disorders, which are termed mood disorders. Chronic stress is generally considered as a key risk factor for the development of a variety of human ailments as it alters the behavior, cardiovascular responses, the immune system, gastrointestinal system (Sutanto and Kloet et al., 1989), and development of many psychological conditions in humans, including major depression (Kendler et al., 1999; 2001).

Stress affects the central nervous system (CNS) leading to the modulation of steroid, catecholamine, peptide and opioid systems. Stress results in activation of the hypothalamic-pituitary-adrenal axis and loss of negative feedback inhibition of the corticosterone (CORT) response resulting in elevated circulating levels of CORT (Herman et al., 1995). The activation and suppression of the HPA axis is modulated by corticosteroids whose action within the CNS are believed to be mediated by two distinct corticosteroid receptor systems: the mineralocorticoid (MR) and glucocorticoid (GR) receptors (McEwen et al., 1995; de Kloet and Sutanto, 1989). MR are localized in septal hippocampal region, anterior hypothalamus and circum ventricular organs while GR are more widely distributed throughout the brain (Raju, 2000, van Eekelen et al., 1987). These persistent elevations in CORT can result in increased susceptibility to depression, disease, and cognitive deficits (McEwen and Sapolsky, 1995; McEwen, 2000;
Holshoer, 2000), as well as dendritic atrophy in the hippocampus and cortex of rodents (McEwen, 1995a,b) Chronic restraint stress causes dendritic atrophy of CA3 pyramidal neurons of the hippocampus (Sunanda et al., 2001). Moreover, in the CNS, MR and GR mediate the different action of corticosteroids. Thus, interaction of corticosterone and/or cortisol (depending upon the species) via MR in the hippocampus is responsible for the tonic influences of the steroid (de Kloet et al., 1999). Stress affects an individual's ability to learn and remember is diverse, there being no simple relationship between stress and learning.

The aforementioned limbic structures as well as the prefrontal cortex have connections to the hypothalamus which plays a crucial role concerning the activation of a physiological stress response induced by endocrinologic changes (Schwabe and Wolf 2013). It has been theorized that the hippocampal formation, which is crucial to memory formation as well as spatial navigation, might also play an important role in stress regulation. The brain regions involved in a physiological stress response extensively overlap with the structures, which are critical for memory processes. These stress effects have been extensively studied with particular focus on hippocampus-dependent memory processing, showing time-dependent effects of the stressor on encoding, consolidation, and retrieval (Roozendaal, 2002; Joëls et al., 2006).

Chronic unpredictable stress (CUS), one of the most clinically relevant stress paradigms in rodents, mimics a number of behavioral characteristics observed in patients with anxiety, depression and related mood disorders. Hence, the un avoidable stressors need to be studied in detail for a better understanding as single stressor is sufficient to affect body system as well as CNS (Manikandan et al., 2006). The current study is focused on the effect of unpredictable stressor was studied by subjecting the rats to single type of stressor /day in a sequence without repeating the stressor for the second day. They included noise stress, immobilization stress, forced swim stress till exhaustion, food deprivation stress for 24 hours day, overnight lights stress, Social isolation stress for 24 hours.

The use of medicinal herbs in industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Shrikumar and Ravi, 2007). A wide interest has been made for researchers using herbal material in identification of the active components and verification of their efficiency. In India, one of the valuable medicinal plant is *Eclipta alba* (L). It popularly known as “King of hairs” used in indigenous system of medicine and Charaka advises taking the juice of *eclipta alba* with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies. (Thorat, et al., 2010).

Eclipta alba commonly known as Bhringraj and Karisilikanni, well as false daisy, and it is called in varies names depending upon the region. *Eclipta alba* is a species of plant in the family Asteraceae which is found a common weed throughout India ascending up to 6000 ft. It is a weed which grows in tropical and subtropical regions all over the world. *Eclipta alba* Hassk is a small evergreen tree with wide geographical and ecological distribution, and its certain parts are used as medicinal materials (Thorat, et al., 2010).

*Eclipta alba* is having important role in the traditional Ayurvedic, Unani systems of holistic health and herbal medicine of the East. *Eclipta Alba* (L.) contains wide range of active principles which includes coumestans (Wagner et al., 1986, Yahara et al., 2006), alkaloids, flavonoids, thiophene derivatives (Yahara et al., 2006); triterpinoidsaponins (Zhao et al., 2001), glycosides, (Shieh and Tsai, 1985), polyacetylenes, wedelolactone (Samiuillia et al., 2003). strychnolactone (Zhang and Guo, 2001). Dithienylacetylene ester (Jain and Singh, 1988), eclip or α-terthienyl aldehyde (Das and Chakravarty, 1991), α-terthienyl-methanol (Han et al., 1998), α-formytherthienyl, Ecliptasaponin C and D (Zhang et al., 1997), new triterpenoidglucosides, eclalbatin, together with α-amyrin, β-amyrin ,ursolic acid, oleanolic acid , and wedylic acid (Upadhyay et al., 2001) , tocontamphytosterol; β glucoside of phytosterol, daucosterol and stigmasteryl-3-O-glucoside (Zhang et al., 1997), stigmasterol (Han et al., 1998) and β-sitosterol (Zhang and Guo, 2001). Alkaloids including ecliptine and nicotine (Amrit Pal Singh et al 2010 Sikhoria et al., 1982). Bio-active steroidal alkaloids, verazine, 20-epi-3-dehydroxy-3-oxo-5, 6-dihydro-4, 5dehydroverazine ecliptalbine, (20R)-4s-hydroxyverazine, 4s-hydroxyverazine, (20R)-25s-hydroxyverazine and 25s-hydroxyverazine have been identified from them ethanolic extract (Abdel Kader et al., 1998).

The important pharmacological activities are Hepatoprotective (Zhang and Guo, 2001), antimicrobial (Arunachalam et al., 2009; Datta et al., 2009), antifungal (Venkatesanand Ravi, 2004), anti-malarial (Bapna et al., 2007), antihyperglycemic (Ananthi et al., 2003), neuroprotective (Banji et al., 2009), hair growth promoter (Datta et al., 2009), neutralized the proteolytic activity, protection against hemorrhagic activity (Pithayanukulet et al., 2007), immunomodulator (Chokotia, et. al., 2013), anticancer Shekokar, S., and Nayak, S. U. (2017) anxiolytic (Monalisa and Swati, 2013), antinociceptive, antiviral, analgesic (Thor et al 2010).

*Eclipta alba* could be demonstrating neuroprotective activity, showed significant cognitive enhancement as well as elevated brain antioxidant enzymes and inhibited cholineresterase activity (Bhaskar and Chintamaneni, 2012). *Eclipta alba* improved learning and memory of rats. Based on these reports *Eclipta alba* was selected for the study.
MATERIALS AND METHODS

Animals: Experimental animals were all healthy and weighed about 140 to 180gms. The animals were reared in the Animal House of the Institute, University of Madras, Taramani, Chennai, India and all the animals were maintained under standard laboratory conditions housed 3 per cage (29 cm X 22 cm X 14 cm) and constant ambient temperature with 12 hours dark photo period, the rats were allowed free access to food and water. Appropriate ethical clearance was obtained for this work from the Institutional Animal Ethical Committee (IAEC no. 01/40/15 dated 26/11/2015) prior to the experiments. Animals were divided into 6 groups, with 6 animals in each group.

STUDY DESIGN

Table 1: Study design.

<table>
<thead>
<tr>
<th>S.No</th>
<th>GROUPS</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>Control treated with \textit{Eclipta alba} (200 mg/kg bw)</td>
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<tr>
<td>3</td>
<td>Acute Unpredictable stress (AUS) (1 day)</td>
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<tr>
<td>4</td>
<td>Chronic Unpredictable stress (CUS) (30 days)</td>
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<tr>
<td>5</td>
<td>Acute Unpredictable stress + Treatment with \textit{Eclipta alba} (Pre treatment (15 days) (AUS+TRT) (200 mg/kg bw)</td>
</tr>
<tr>
<td>6</td>
<td>Chronic unpredictable stress (30 days) + pre Treatment with \textit{Eclipta alba} (15 days) and treated during stress exposure (CUS+TRT) (200 mg/kg bw)</td>
</tr>
</tbody>
</table>

Fig 1: Chronic Unpredictable Stress Paradigm.

Unpredictable Stress Paradigm:
- Noise stress(100 dB) for 4hrs
- Immobilization stress for 4hrs
- Forced swim stress for 15 min
- Food deprivation stress for 24hours/day
- Overnight lights on stress for 12 hours
- Social isolation stress for 24hours

In acute stress, the stressors are exposed one followed by one.
In chronic stress, single type of stress per day.

(i) Orientation session: This session was to familiarize the rats with the T- maze. Rats are semi starved for 24h and allowed to explore the T-maze for 15 min. Rats were placed in the start box for 30 seconds. Sliding door was opened to allow rats to explore the T-maze for 15 min and to eat food pellets in each goal area. Rats were then returned to the start box.

(ii) Learning Performance Test: After the first day of orientation the rats were trained for the rewarded alternation task. Learning performance test followed the same procedure as the orientation session except that one arm is baited. The rat has to alternate the arms for the reward. The placement of the reward in the subsequent trial will be opposite to the rats previous arm entry. Each rat received ten trials per day. The inter-trial interval was 30 seconds, during which the maze was cleaned with 70% alcohol to remove odor cues. The number of days each rat took for 80% correct choice (Eight correct entries to the baited arm out of ten trials) and the time taken to reach the goal area was noted.

(iii) Retention test: Two days after the last training session, memory retention test was carried out. Rats were given ten trials continuously, with an inter-trial interval for 30 seconds. Numbers of errors i.e., entry into the non-rewarded arm is recorded in ten trials were noted.

STATISTICAL ANALYSIS ONE WAY ANOVA

All the results were analyzed by SPSS version 20. All the data were expressed as mean ± SD, analyzed by one way ANOVA followed by Tukey’s multiple comparison test and the significance level was fixed at <0.05

RESULTS

LATENCY

![Fig. 2: indicates significance compared with control group, # - indicates significance compared with CUS group, @ - indicates significance compared with AUS group, Significance level was fixed P<0.05.](image)

Latency is the time duration taken to complete a session. In this study acute and chronic unpredictable stress exposed animals showed a significant increase in the time to complete the task i.e. in reaching the baited arm.
when compared with control animals. Chronic stress exposed animals showed a significant increase in the latency when compared with acute stress exposed animals. Stress exposed treated animals showed a significant decrease in the latency when compared with its respective stressed groups, but their latency in completion the task was higher than the control animals. No significant change was noted between control and *Eclipta alba* alone treated animals.

### NUMBER OF CORRECT CHOICE

![Graph showing number of correct choice](image)

**Fig. 3:** indicates significance compared with control group, # - indicates significance compared with CUS group, @ - indicates significance compared with AUS group, Significance level was fixed P<0.05.

Correct choice is the percentage of animals entering the correct baited arm. In this study acute and chronic unpredictable stress exposed animals showed a significant decrease in the percentage of correct choice when compared with control animals. Chronic stress exposed animals showed a significant decrease in the correct choice percentage when compared with acute stress exposed animals. Stress exposed treated animals showed a significant increase in the correct choice percentage when compared with its respective stressed groups, but their correct choice percentage was lower than the control animals. No significant change was noted between control and *Eclipta alba* alone treated animals.

### NUMBER OF DAYS TAKEN TO ATTAIN 90% CORRECT CHOICE

![Graph showing number of days taken to attain 90% correct choice](image)

**Fig. 4:** indicates significance compared with control group, # - indicates significance compared with CUS group, @ - indicates significance compared with AUS group, Significance level was fixed P<0.05.

Acute and chronic unpredictable stress exposed animals showed a significant increase in the number of days in attaining 90% correct choice when compared with control animals. Chronic stress exposed animals showed a significant increase in the number of days when compared with acute stress exposed animals. Stress exposed treated animals showed a significant decrease in the number of days when compared with its respective stressed groups, but the number of days taken by these animals to reach 90% correct choice was higher than the control animals. No significant change was noted between control and *Eclipta alba* alone treated animals.

### DISCUSSION

Stresses of life can precipitate number of mental illnesses. Modern day life style leads to numerous stressful conditions. Moreover their prevalence is increasing day by day. Sandi ND Pinelo-Nava, 2007 explained that Stress might influence learning and memory processes by suppression of adult neurogenesis or by affecting neurochemical systems (for example, catecholamines and glucocorticoids). Stress is known to cause memory impairment. Neural basis of such impairment is the neuronal injury in the hippocampal region due to excitotoxicity, alterations in the neurotransmitter levels in the brain regions. Alternatively neuronal damage in this region may be mediated through increased glucocorticoids. Stress results in increased oxidative stress and decreased antioxidant defense status in brain, which may form the basis for decreased memory (Kumar et al., 2009). Increased central noradrenergic activity can be associated with impairment of prefrontal cortex functions involved in working memory performance (Ramos & Amsten, 2007) and could play a role in the lower accuracy observed in the stressed group.

Due to the various side effects of allopathic drugs used for treatment of these diseases, there is continuous search for alternative treatment. So it is prudent to look for options which are efficacious and safer. Indigenous systems of medicine including natural herbs are time tested way of treatment. Herbal medicines emphasize the prevention of the diseases, rejuvenation of our body systems, maintain balance and harmony and extend the life span (Himani et al., 2013).

*Eclipta alba* was given orally at the dose of 200 mg/kg body weight as a single dose for 30 consecutive days to group, @ - indicates significance compared with AUS group, Significance level was fixed P<0.05.
the test rats and exposed of various kinds of unpredictable stress, each stress per day at various frequency range and assessed by T-maze.

Results indicate that *Eclipta alba* treated rats showed a high percentage of correct response to reach the goal when compared to the acute and chronic stress groups, there is an impaired spatial learning ability in acute and chronic stress groups, which may be due to stress induced damage to hippocampus. Acute stress + *Eclipta alba* treated group, chronic unpredictable stress + *Eclipta alba* treated group showed an increased correct response, decrease day and time taken to reach the goal when compared to acute stress and chronic unpredictable stress alone groups. This shows that memory enhancing property of *Eclipta alba* in stress induced memory deficits in wistar albino rats.

Previous investigations have revealed that herbal supplementation can significantly control cognitive and motor neuron dysfunction. Similarly, medicinal plants and their bioactive constituents can improve behavioral (motor and cognitive behavior), neuronal signaling and anti-inflammatory effects (Mithun et al., 2011). In the present study supplementation of *Eclipta alba* has significantly enhanced the memory in both acute and chronic unpredictable stress exposed animals.

The aqueous and hydroalcoholic extracts of *Eclipta alba* have been evaluated for sedative, muscle relaxant, anxiolytic, nootropic, and antistress activities at doses of 150 and 300 mg/kg. The findings indicated nootropic activity of the aqueous extract (300 mg/kg, p.o.) and its hydrolyzed fraction (30 mg/kg) (Thakur and Mengi 2005). Otiila et al. (2007) examined aqueous extract of leaves of *Eclipta alba* for its memory enhancing quality and the results revealed significant improvement of retrieval memory. Abana, an Ayurvedic herbomineral preparation containing *Eclipta alba*, was also investigated for effects on memory in rats. Extracts of *Eclipta alba* (50, 100 and 200 mg/kg) produced a dose-dependent improvement in memory scores of young and aged rats (Milind and Vasudevan, 2007). Furthermore, it reversed the amnesia induced by scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.). It may prove to be a useful remedy for the management of Alzheimer’s disease (Vasudevan and Milind, 2008). In the present study also there is a significant increase in the memory enhancement upon treatment with *Eclipta alba*.

*Eclipta alba* found to be having antioxidant activity. The present study revealed that *Eclipta alba* enhances memory while observed in T-maze. *Eclipta alba* has antioxidant activity and also decreased brain acetylcholinesterase enzyme level and thereby elevated acetylcholine concentration in the brain and ultimately improved memory in acute and chronic stress induced groups. Since *Eclipta alba* is easily available perennial plant and is already under human consumption. It will be a better choice to be an antidote for stress induced memory impairment.

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**COMPETING INTERESTS**

No competing interest declared.

**AUTHOR CONTRIBUTIONS**


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