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NEUROPROTECTIVE ROLE OF HERB *ECLIPTA ALBA* LINN. ON ACUTE AND CHRONIC UNPREDICTABLE STRESS INDUCED BEHAVIORAL, BIOCHEMICAL AND NEURO-TRANSMITTER ALTERATION IN WISTAR MALE ALBINO RAT BRAIN

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Keywords:

Eclipta alba, CUS, Neurotransmitters, Cognition, Antioxidants

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ABSTRACT: Chronic stress is a risk factor for the development of many psychopathological conditions in humans, including major depression and anxiety disorders and cognitive dysfunction. It is therefore important to focus on identifying possible mechanism to prevent these stress-induced disorders. Therefore, current study was designed to investigate the effect of *Eclipta alba* on chronic unpredicted stress (CUS) induced behavioral dysfunction, oxidative stress and neurotransmitter levels in the discrete regions of rat brain. Animals were subjected to acute and chronic unpredictable stress for 30 days, and the effect of *Eclipta alba* was studied in acute and chronic stressed animals during which 200 mg/kg *Eclipta alba* was administered orally. Our Results revealed that stressed animals performed poorly in Novel object recognition task (NORT). *Eclipta alba* alleviated behavioral dysfunction in chronically stressed animals and also significantly increased antioxidant levels such as SOD, Catalase, total thiol and decreased oxidative stress markers such MDA, nitric oxide and hydrogen peroxide in discrete brain regions as compared to CUS. Further increased catecholamine and decreased 5HT due to chronic stress in the hippocampus, prefrontal cortex and corpus stiatum was normalized with the treatment of *Eclipta alba*. In conclusion, *Eclipta alba* can efficiently prevent stress induced neurological complications by its antioxidant and efficiently regulating altered neurotransmitters and preventing DNA Damage.

INTRODUCTION: Inability to counteract the excessive production of reactive oxygenated species by antioxidants leads to perturbation of cell redox balance. Stress causes damage to the mitochondrial membranes and protein structure which in turn enhances reactive oxygenated species generation, leading to DNA impairment and apoptosis [1].

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Converging evidence shows that oxidative stress is a critical mediator of cognitive impairments such as those found in Alzheimer's disease, and that treatment with dietary antioxidants can reverse these deficits. Stress induced neuronal damage associated with behavioral changes especially cognitive dysfunction is well reported [2, 3]. The brain and nervous system are prone to oxidative stress, and are inadequately equipped with antioxidant defense systems to prevent 'ongoing' oxidative damage. Neurons are vulnerable to lipid peroxidation-induced damage because the major part of neuronal cell membrane is polyunsaturated fatty acids that are substrate for reactive oxygen species [4].

Mounting evidence suggests that acute and chronic stress, especially the stress-induced release of glucocorticoids, induces changes in glutamate neurotransmission in the prefrontal cortex and the hippocampus, thereby influencing some aspects of cognitive processing. Depending on the age of the animal at the time of exposure, and the duration and type of stressor experienced, stress also has marked and often divergent effects on learning and memory [4, 5] 15 days of noise stress in wistar albino rats has shown to alter neurotransmitters epinephrine, norepinephrine, 5HT and dopamine [6] in the same study altered neurotransmitters were normalized with *Ocimum sanctum* Linn. in discrete regions of brain. Since ancient times many herbs have been used in Indian system of traditional medicine.

Eclipta alba (L.) Hassk. belongs to the Asteraceae family. It is commonly known as false daisy in English, bhringraj in Hindi and Karisslaankanni in Tamil. In ayurvedic medicine, the leaf extract is considered a powerful for the hair growth [7] Herb is reported to possess antileptospiral, antioxidant [8] neuroprotective, memory enhancing, anti-acetyl cholinesterase and antistressor activity [9] Hepatoprotective [10] Lipid lowering activity [11] the present study deals with the evaluation of potential therapeutic role of *Eclipta alba* linn. in combating biochemical, behavioral and neurotransmitter alteration induced by acute and chronic unpredictable stress.

MATERIALS AND METHODS:

Collection of Plant and Preparation of Extract: 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.

Animals: Healthy male Wistar albino rats weighing between 180 - 220 gms were used for the study after obtaining approval from Institutional Animal Ethical Committee (IAEC No: IAEC No-01/25/2014) all the animals were maintained under standard laboratory conditions and were allowed to have food and water *ad libitum* at Central Animal House Facility, University of Madras, Taramani.

Experimental Design:

Animal Grouping: Experimental Animals were randomly divided into 6 groups (n = 6) Control, Acute unpredictable stress (AUS), AUS+ EEEA, Chronic Unpredictable Stress, CUS+EEEA, EEEA Treated alone

Induction of Stress [12] (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: CU STRESS PROTOCOL

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 min
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 on	12 hrs

For acute unpredictable stress - Noise stress, cage isolation and overnight illuminated lights on stressors were applied on single day followed by which animals were considered for further study.

Dosage and Route of Administration: EEEA was dissolved in warm normal saline (0.9% NaCl). Dosage - 200 mg/kg body weight [13] was administered through the oral route. Drug was administered 1 hr before induction of stress in case of CUS+EA group.

HPLC: Determination of neurotransmitter concentrations. The various brain biogenic amines in discrete regions of the rat brain were estimated by the method of [14] Wagner *et al.*, [8] The rats were sacrificed by cervical dislocation. After sacrifice, the brain was rapidly removed, and the cerebral cortex, hippocampus and corpus striatum were dissected on an ice-cold plate [15]. Concentrations of nor epinephrine (NE), epinephrine (E), dopamine (DA), and 5-hydroxytryptamine (serotonin, 5-HT) were measured by high performance liquid chromatography (HPLC) coupled with

electrochemical detection (ECD). All brain regions were homogenized with perchloric acid. The mobile phase containing citric acid, di sodium hydrogen orthophosphate, EDTA, octane-1-sulphonic acid sodium salt, and 14% methanol and was adjusted to the pH of 4.0 using di sodium hydrogen orthophosphate. Homogenates were centrifuged (12,000 rpm, 4 °C) in a refrigerated centrifuge for 2 min, and then the internal standard dihydroxybenezylamine (DHBA) was added to the supernatant of brain homogenate and again centrifuged at 12,000 rpm for 20 min.

The supernatant was filtered with 0.22-µm membrane filter, and 20 µl of sample was injected into the Rheodyne injector (Cotati, CA, USA) of the HPLC system, which is connected to an isocratic pump (Model 501; Waters Association, Millipore, MA, USA) and reverse phase column LiChroCART RP-18, for separation of indole amines and catecholamine. The reaction products were detected with an electrochemical detector (Model 460, Waters), which was coupled to the HPLC system and set at a potential of +0.60 V for the detection of monoamine neurotransmitters. The flow rate was maintained at 0.8 ml/min. Neurotransmitters were quantified using a C-R8A data processor (Shimadzu, Kyoto) and expressed as nano grams of neurotransmitter per gram of wet weight of brain tissue.

Biochemical Assays: Biochemical determinations Protein was estimated as per the method described by Lowry *et al*., (1951) [16]. Lipid peroxidation was determined in the tissue samples as described by Ohkawa *et al*., (1979) [17]. Nitric oxide (NO) levels were measured as total nitrite + nitrate levels with the use of the Griess reagent by the method of Moshage, (1995) [18]. Protein thiol by Sedlack and Lindsay, (1968) [19] was determined. Superoxide dismutase (SOD) according to Marklund and Marklund, (1974) [20] and catalase (CAT) according to the method of Sinha, (1972) [21]. The activity of glutathione peroxidase (GPx) was estimated by the methods of Rotruck *et al*., (1973) [22]. Reduced glutathione (GSH) in the tissue samples was estimated by the method of Moron *et al*., (1979) [23]. The Vitamin C (ascorbic acid) content in the tissue was determined according to the method of Omaye *et al*., (1979) [24]. Vitamin E by the method of Desai [25], GR [26], protein carbonyl by the method of Levine [27], H_2O_2 [28].

DNA Fragmentation: 100 mg of whole brain tissue was homogenized and DNA was isolated. DNA were resolved by electrophoresis through a 0.8% agarose gel and stained with ethidium bromide. The resolved fragments of DNA in the agarose gel were scanned with a Gel Doc image scanner (Bio-Rad, USA).

Behavioral Assessment: Novel Object Recognition Task [29].

RESULTS:

SOD Activity Fig. 1: The *Eclipta alba* treated control rats did not deviate from controls. In the acute stressed animals the SOD level was increased markedly from controls in all the brain regions namely cerebral cortex (df 5, F = 248), hippocampus (df 5, F = 1218), corpus striatum (df 5, F = 558), hypothalamus (df 5, F = 573), and cerebellum (df 5, F = 256).

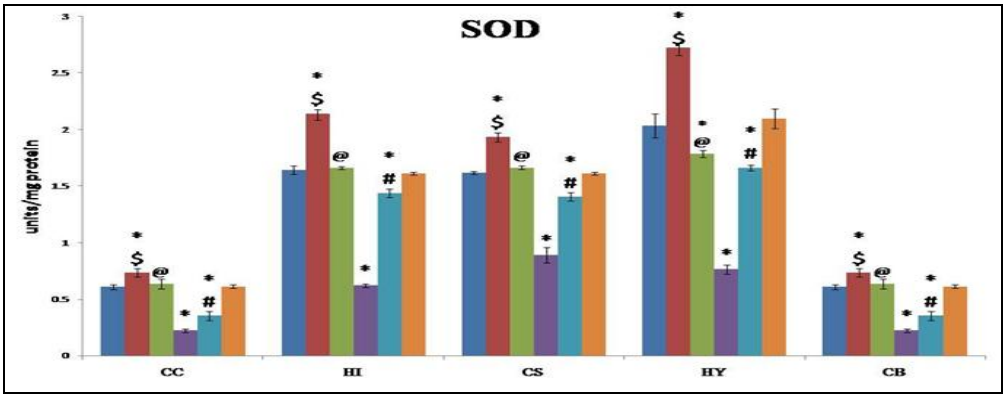

FIG. 1: EFFECT OF *ECLIPTA ALBA* ON SUPEROXIDE DISMUTASE (SOD) LEVEL IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AUS

However, chronic stress group showed a significant decrease in the SOD levels indicating oxidants and antioxidants imbalance irrespective of brain regions. In acute stress groups *Eclipta alba* treatment could decrease the SOD activity similar to control except hypothalamus where SOD activity showed further decrease from control level. In chronic stresses animals treated with *Eclipta alba* showed a marked increase in SOD activity from stressed animals.

Catalase Activity Fig. 2: The *Eclipta alba* treated control rats did not deviate from controls. However, in the acute stressed animals the catalase level was decreased from that of controls in all the brain regions namely cerebral cortex (df 5, F = 36), hippocampus (df 5, F = 232), corpus striatum (df 5, F = 201), hypothalamus (dfdf 5, F = 30), except cerebellum (df 5, F = 126) where it was found to be increased $p < 0.05$, the catalse activity was found to be further decreased in Chronic stress in all the brain regions. In acute stress groups *Eclipta alba* treatment normalized the catalase activity. In the chronic stressed animals treated with *Eclipta alba* showed a marked increase from stressed animals. Though they still showed a significant decrease from control levels.

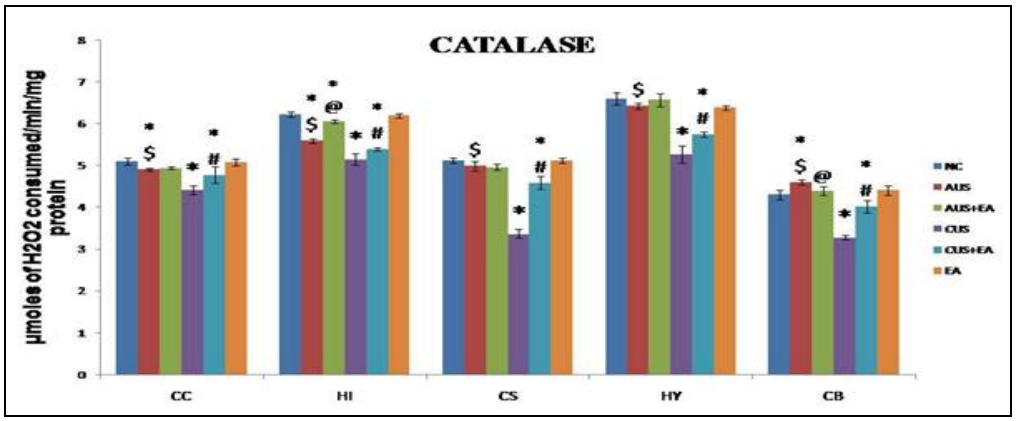

FIG. 2: EFFECT OF *ECLIPTA ALBA* ON CATALASE ACTIVITY LEVEL IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

GPX Fig. 3: The *Eclipta alba* treated control rats did not deviate from controls in cortex (df 5, F = 362), hippocampus (df 5, F = 705), corpus striatum (df 5, F = 490), hypothalamus (df 5, F = 239), cerebellum (df 5, F = 310). In the acute stress as well as chronic stress the GPX level was increased significantly in all the regions $p < 0.05$, however chronic stress showed marked increase than acute $p < 0.05$ indicating oxidants and antioxidants imbalance irrespective of brain regions.

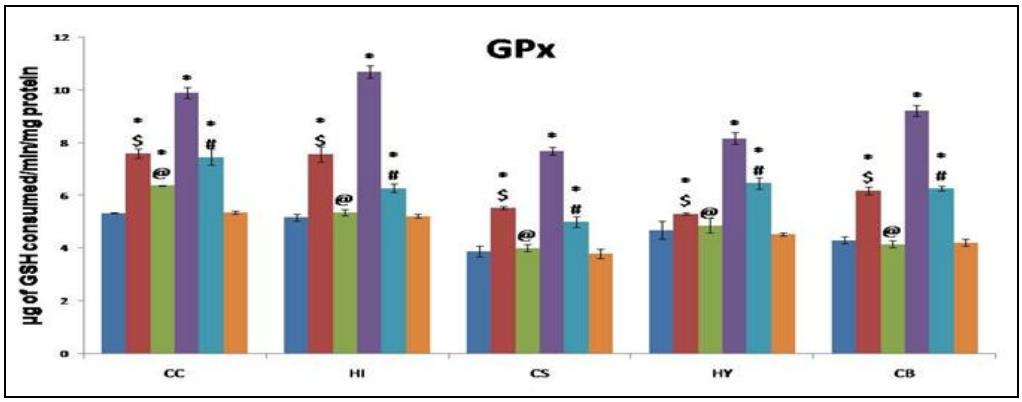

FIG. 3: EFFECT OF *ECLIPTA ALBA* ON GPx IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

In Acute stress treated animals GPx levels were brought back to control levels except in Cortex where it was still high as compared with control. Whereas chronic Stress groups treated with *Eclipta alba* showed significant improvement.

GSH Fig. 4: *Eclipta alba* treated control did not deviate from control in cortex (df 5, F = 119), hippocampus (df 5, F = 36), corpus striatum (df 5, F = 79), hypothalamus (df 5, F = 59), and cerebellum (df 5, F = 20). In the acute stress as well as chronic stress the GSH level was decreased significantly in all the regions $p < 0.05$, however chronic stress showed marked decrease than acute $p < 0.05$. AUS treated group did not show any change compared to control indicating effectiveness of EEEA in AUS treated group whereas chronic Stress groups treated showed significant improvement.

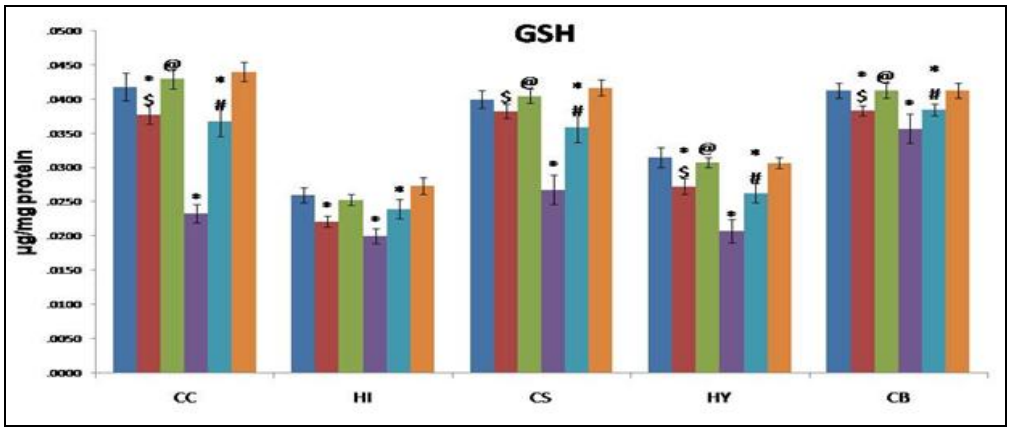

FIG. 4: EFFECT OF *ECLIPTA ALBA* ON GSH IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Lipid Peroxidation Fig. 5: The drug treated control did not deviate from control in cortex (df 5, F = 3004), hippocampus (df 5, F = 1074), corpus striatum (df 5, F = 507), hypothalamus (df 5, F = 1501), and cerebellum (df 5, F = 821). In the acute stress as well as chronic stress the amount of MDA formed was increased significantly in all the regions $p < 0.05$, however chronic stress showed marked increase than acute $p < 0.05$ indicating increased lipid peroxidation irrespective of brain regions whereas respected Stress groups treated with 200 mg/kg bw EEEA showed significant improvement though was not normalised to control levels this indicates the significant imbalance in oxidants and antioxidants.

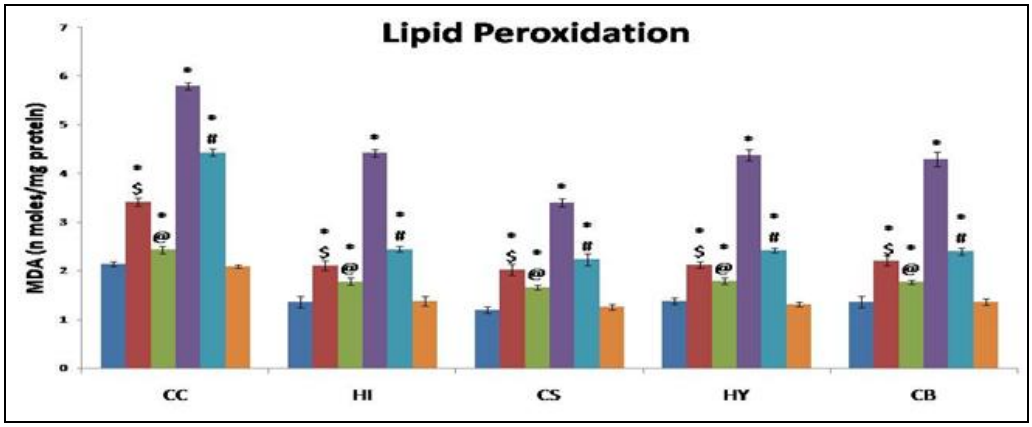

FIG. 5: EFFECT OF *ECLIPTA ALBA* ON AMOUNT OF MDA FORMED IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Vitamin C Fig. 6: The treated group did not deviate from control in Cortex (df 5, F = 167), Hippocampus (df 5, F = 76), Corpus Striatum (df 5, F = 289), Hypothalamus (df 5, F = 284), Cerebellum (df 5, F = 297). In the acute stress as well as chronic stress the Vitamin C level was decreased significantly in all the regions $p < 0.05$, however chronic stress showed marked decrease than acute $p < 0.05$. whereas respected Stress groups treated with 200 mg/kg bw EEEA showed

significant improvement though was not normalised to control levels except in cortex, Cerebellum and corpus striatum of AUS treated group where it was brought to the control levels.

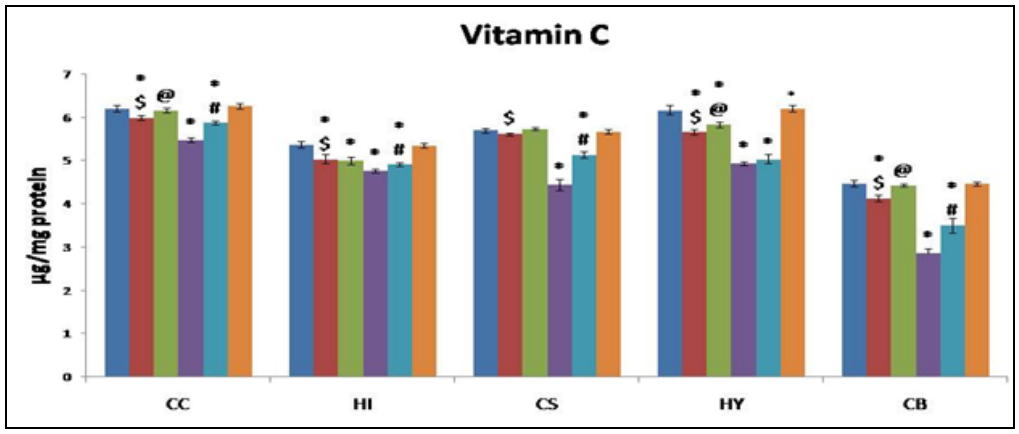

FIG. 6: EFFECT OF *ECLIPTA ALBA* ON AMOUNT OF VITAMIN C IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

GR Fig. 7: The drug treated control did not deviate from control in Cortex (df 5, F = 409), Hippocampus (df 5, F = 61), Corpus Striatum (df 5, F = 1142), Hypothalamus (df 5, F = 114), Cerebellum (df 5, F = 148). In the acute stress as well as chronic stress the GR level was decreased significantly in all the regions $p < 0.05$, however chronic stress showed marked decrease than acute $p < 0.05$ whereas respected Stress groups treated with EEEA showed significant improvement.

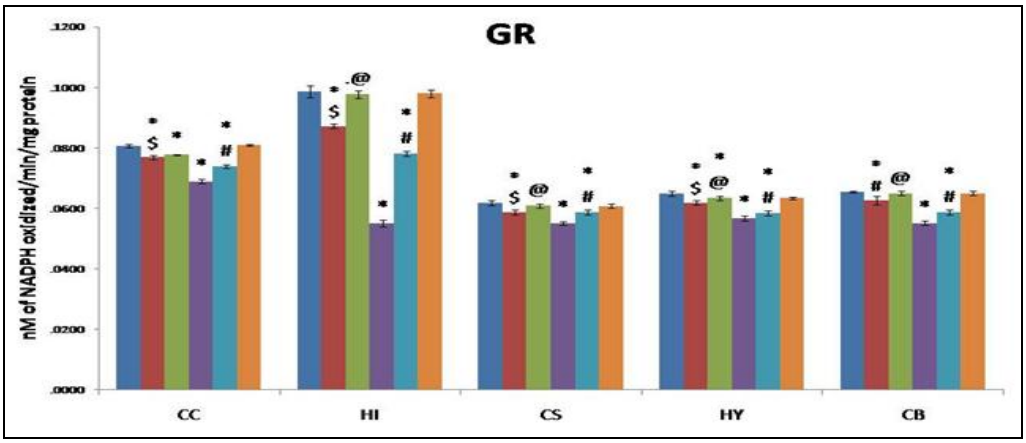

FIG. 7: EFFECT OF *ECLIPTA ALBA* ON GLUTATHIONE REDUCTASE ACTIVITY IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Nitric Oxide Fig. 8: The treated group did not deviate from control *in Cortex (df 5, F = 46), Hippocampus (df 5, F = 56), Corpus Striatum* (df 5, F = 53), Hypothalamus (df 5, F = 271), Cerebellum (df 5, F = 235). In the acute stress as well as chronic stress the nitric oxide levels was increased significantly in all the regions $p < 0.05$, however chronic stress showed significant increase than acute stress $p < 0.05$ irrespective of brain regions whereas respected Stress groups treated showed significant improvement.

H_2O_2 Fig. 9: The treated group did not deviate from control in Cortex (df 5, F = 8117), Hippocampus (df 5, F = 319), Corpus Striatum (df 5, F = 1075), Hypothalamus (df 5, F = 2702), Cerebellum (df 5, F = 2247). In the acute stress as well as chronic stress the hydrogen peroxide levels was increased significantly in all the regions $p < 0.05$, however chronic stress showed significant increase than acute stress $p < 0.05$. Whereas respected Stress groups treated showed significant improvement.

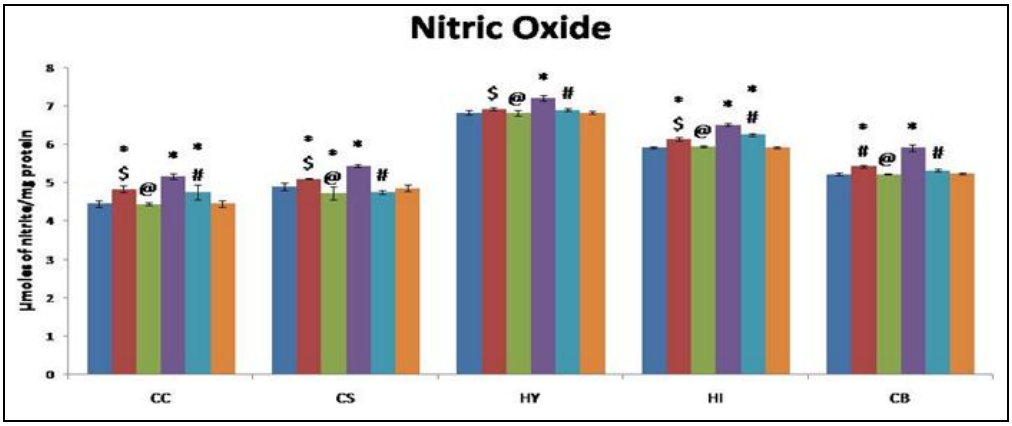

FIG. 8: EFFECT OF *ECLIPTA ALBA* ON NITRIC OXIDE LEVELS IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

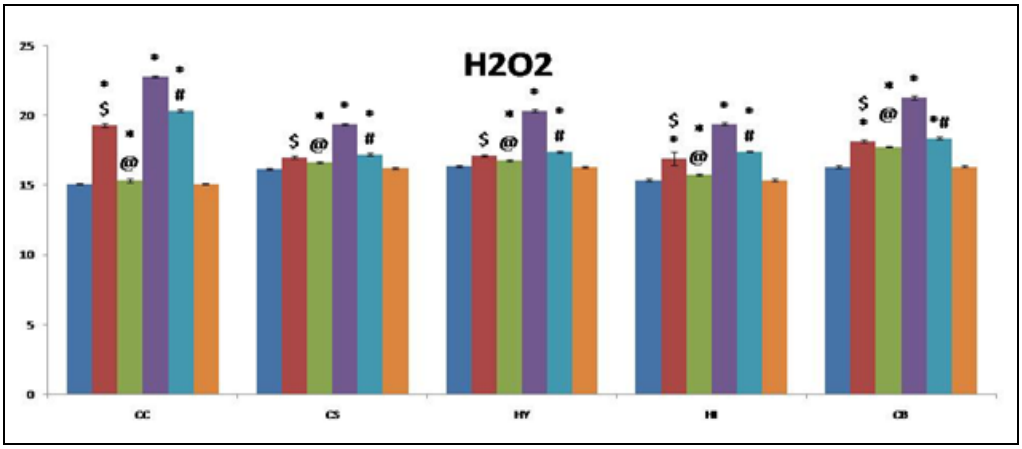

FIG. 9: EFFECT OF *ECLIPTA ALBA* ON H_2O_2 LEVELS IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Protein Thiol Fig. 10: The treated group did not deviate from control in any brain region such as cortex (df 5, F = 866), hippocampus (df 5, F = 448), corpus striatum (df 5, F = 557), hypothalamus (df 5, F = 27), cerebellum (df 5, F = 297). In the acute stress as well as chronic stress the protein thiol level was decreased significantly in all the regions $p < 0.05$, however chronic stress showed marked decrease than acute $p < 0.05$ whereas respected Stress groups treated showed significant improvement.

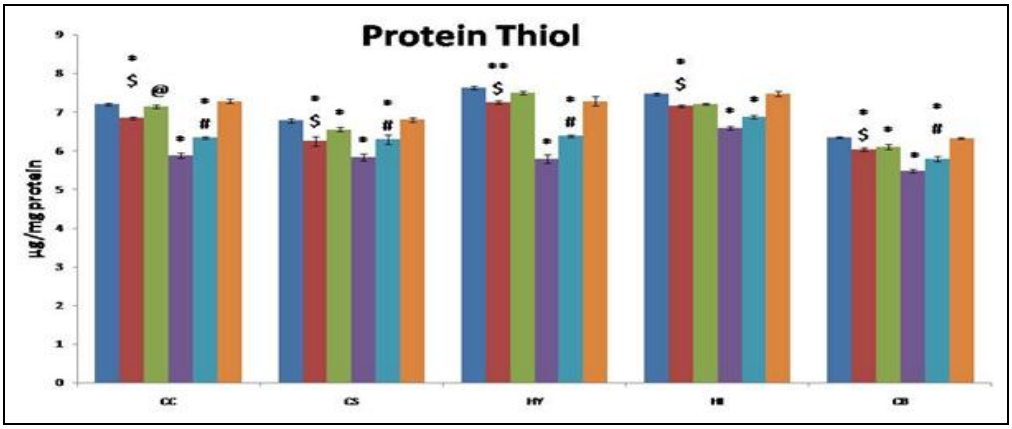

FIG. 10: EFFECT OF *ECLIPTA ALBA* ON PROTEIN THIOL LEVELS IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Vitamin E Fig. 11: The treated did not deviate from control in Cortex (df 5, F = 866), Hippocampus (df 5, F = 138), Corpus Striatum (df 5, F = 1607), hypothalamus (df 5, F = 1253), cerebellam (df 5, F = 1793). In the acute stress as well as chronic stress the Vitamin E level was decreased significantly in all the regions $p < 0.05$, however chronic stress showed marked decrease than acute $p < 0.05$ irrespective of brain regions whereas respected Stress groups treated showed significant improvement.

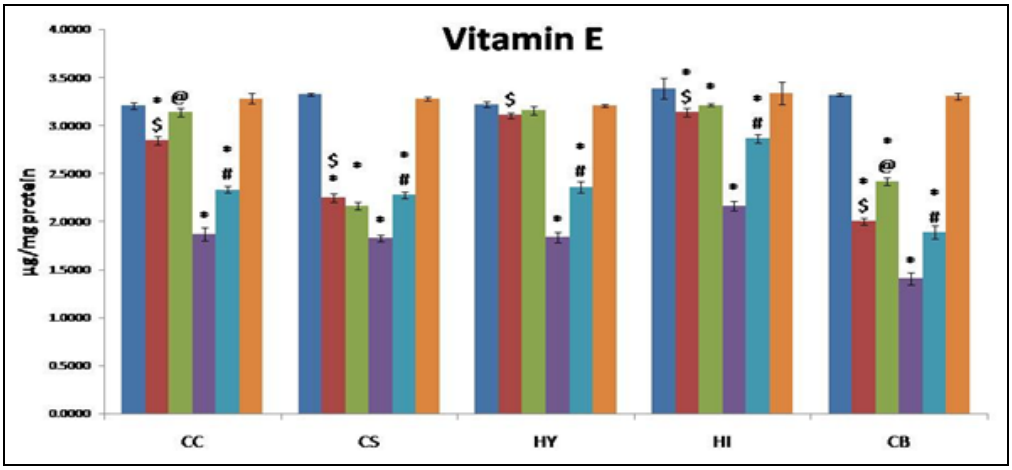

FIG. 11: EFFECT OF *ECLIPTA ALBA* ON VITAMIN E LEVELS IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Protein Carbonyl Fig. 12: The drug treated control did not deviate from control in cortex (df 5, F = 1483), hippocampus (df 5, F = 232), corpus striatum (df 5, F = 507), hypothalamus (df 5, F = 182), cerebellum (df 5, F = 203). In the acute stress as well as chronic stress the Protein carbonyl levels was increased significantly in all the regions $p < 0.05$, however chronic stress showed significant increase than acute stress $p < 0.05$ irrespective of brain regions whereas respected Stress groups treated EEEA showed significant improvement though was not normalized to control levels.

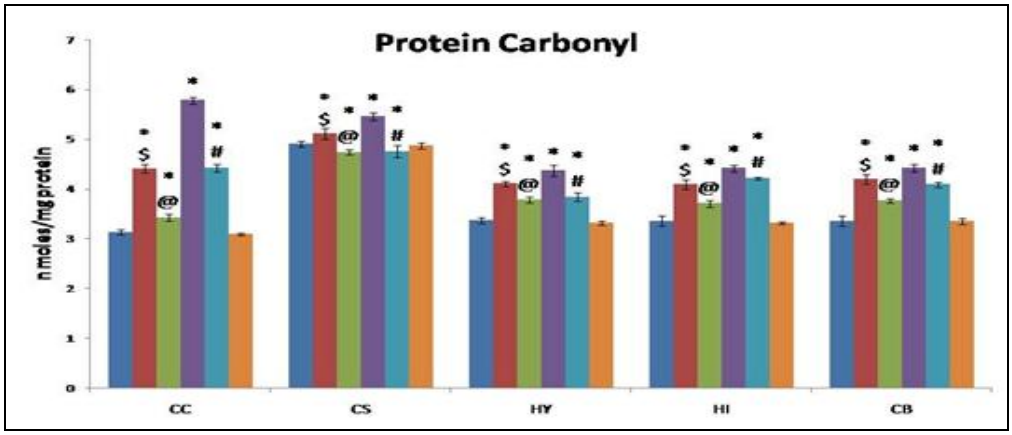

FIG. 12: EFFECT OF *ECLIPTA ALBA* ON PROTEIN CARBONYL LEVELS IN CUS INDUCED ALTERATION IN DISCRETES BRAIN REGIONS. Significance at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AU

Plasma Corticosterone levels Fig. 13: Plasma corticosterone **Fig. 13** indicates the role of Ethanolic extract of *Eclipta alba* (EEEA) on acute and chronic unpredictable stress (CUS) induced change in plasma corticosterone levels. (df 5, F-166.972) Animals Exposed to 1 day as well as 30 days Unpredictable stress showed increase in the corticosterone level. Acute stress as compared to chronic stress showed significant increase $p < 0.05$. Upon treatment AUS+EA and CUS+EA have shown significant decrease in the plasma corticosterone levels owing to its antistressor

activity. EA treated group did not deviate statistically from control though decrease is observed.

Novel Object Recognition Task: NORT Fig. 14a-14c: Exploration of Novel object is an innate behavior of rats, when we subjected all four group rats to the NOR Task, we observed difference in the exploration behaviour. ANET (absolute novel exploratory behavior) (df-5, F-209.490) Animals exposed to CUS spent considerably less time exploring the novel object with $p < 0.05$ when compared with control and other groups.

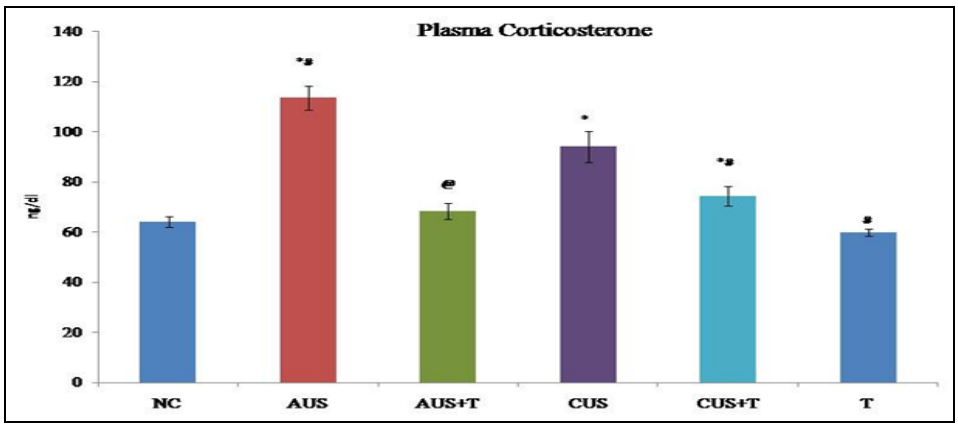

FIG. 13: EFFECT OF EA ON PLASMA COTICOSTERONE IN WISTAR ALBINO RATS EXPOSED TO AUS AND CUS significance at p<0.05, *- compared with control, # - CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AUS

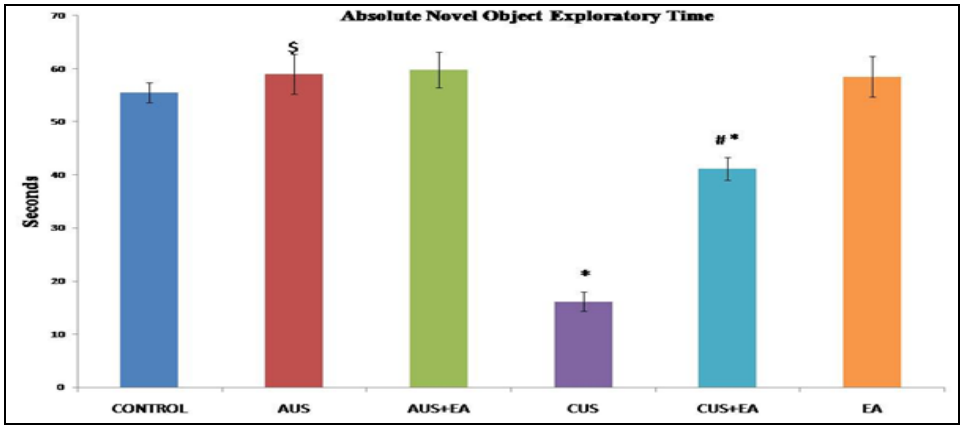

FIG. 14A: NOVEL OBJECT EXPLORATORY TIME – TOTAL TIME SPENT EXPLORING THE NOVEL OBJECT. All the values are represented as mean ± SD. Significance set at $p < 0.05$, *- compared with control, @- AUS compared with AUS+EA, #- CUS compared with CUS+EA, $- AUS compared with AUS+EA

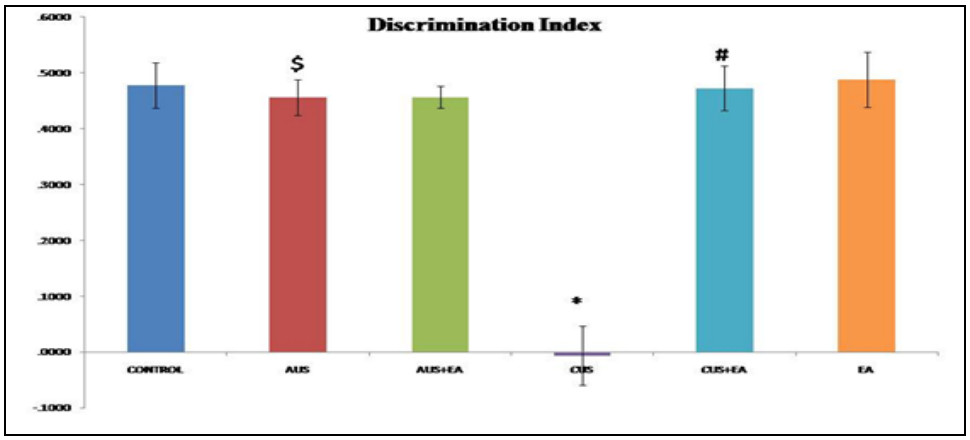

FIG. 14B: DISCRIMINATION INDEX EXPLORATION OF NOVEL OBJECT (SEC) – EXPLORATION OF FAMILIAR OBJECT (SEC) / TOTAL TIME (BOTH OBJECTS IN SECONDS). All the values are represented as mean ± SD. Significance set at $p < 0.05$, *- compared with control, @- AUS compared with AUS+EA, #- CUS compared with CUS+EA, $- AUS compared with AUS+EA

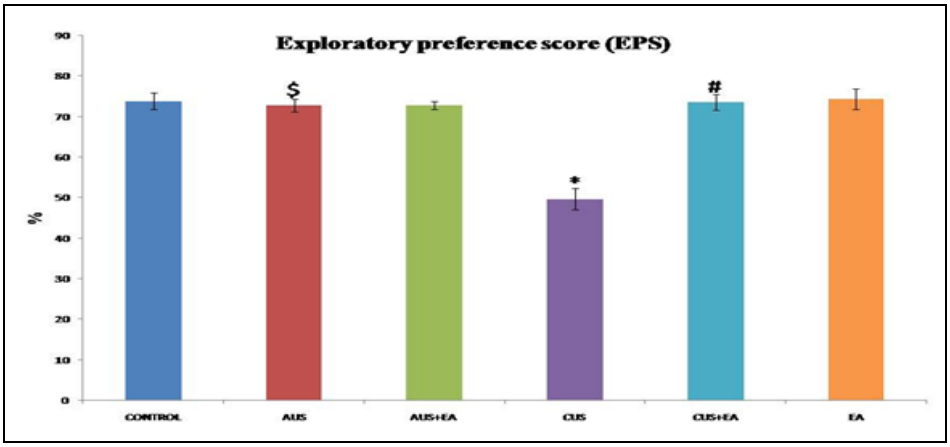

FIG. 14C: EXPLORATORY PREFERENCE SCORE IS EXPRESSED IN PERCENTAGE, *i.e.* TIME SPENT EXPLORING NOVEL OBJECT DIVIDED BY TOTAL TIME SPENT WITH BOTH OBJECT × 100. All the values are represented as mean ± SD. Significance set at $p < 0.05$, *- compared with control, @- AUS compared with AUS+EA, #- CUS compared with CUS+EA, $- AUS compared with AUS+EA

Stressed rats showed less preference for Novel object as compared with control and other groups and upon treatment there was significant change, Preference towards novel object was significantly more as assessed by Exploratory preference score(df-5, F-138.46) Discrimination Index (DI) (df-5, F-138.257) 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. Acute stress animals as well as AUS+EA did not deviate much from control.

Neurotransmitter Estimation in Different Brain Regions:

Epinephrine Table 1: levels in the hippocampus (df-5,F-118.285),Corpus striatum (df-5, F-402.88), PFC(df-5, F-802.362) were invariably increased in acute as well as Chronic stress group as compared to control ($p < 0.05$), respective treatment group showed decrease from that of stress group though was not normalized to control except in prefrontal cortex. EEEA treated group did not deviate from that of control.

TABLE 1: EFFECT OF *ECLIPTA ALBA* ON CATECHOLAMINES EPINEPHRINE AND NOR - EPINEPHRINE IN CU STRESS INDUCED NEUROTRANSMITTER ALTERATION IN DISCRETE REGION OF BRAIN

Groups	Epinephrine			Nor - Epinephrine		
	PFC	CS	HI	PFC	CS	HI
Control	6687_3 ± 214_52	1882_167 ± 103_82	154_33 ± 17_6	7851_5 ± 233	9813 ± 311_51	3422_3 ± 140_8
AUS	9626_33 ± 344_15*$	4601_77 ± 315_5*	1903_8 ± 83_92*	10043 ± 213_4*	8514_5 ± 322_39*	5793_16 ± 117_76*
AUS + EA	6766_33 ± 671_08*@	2538_6 ± 107_55*@	878_5 ± 60_31*@	8515 ± 265_94$	8067 ± 437_94$	2502 ± 192_82
CUS	9336_61 ± 293_157*	5527_83 ± 225_72*	2077_66 ± 115_7*	9185_66 ± 141_25*	8558_05 ± 303_93*	5447 ± 160_343*
CUS + EA	6509_3±219_3#	3159_5±73_87*#	1374_3±86_68*#	8109_5±199_28#	8816_5±232_68#	3481±111_06#
EA	5997±73_93	2144±106_079	120_1667±14_66*	7670_16±112_35	9703_33±320_52	3453_16±205_69

Data are expressed as mean ± SD. Significance set at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AUS

Norepinephrine Table 1: levels in the hippocampus (df-5, F-246.436), PFC (df-5, F-802.362) were invariably increased in acute as well as Chronic stress group as compared to control ($p < 0.05$), whereas in Corpus striatum (df 5, F-688.484) decrease in the levels were observed, respective treatment group in Hippocampus, corpus striatum and PFC showed decrease from that of stress and was normalized to control. EEEA treated group did not deviate from that of control.

Dopamine Table 2: levels in the hippocampus (df 5, F-1570.40), Corpus striatum (df 5, F-2795.706), PFC(df 5, F-293.9) were invariably increased in

both acute as well as Chronic unpredictable stress group as compared with control ($p < 0.05$). indicating altered neurotransmitter levels in response to stress, respective treatment group showed decrease from that stress group though was not normalized to control. EEEA treated group did not deviate from that of control.

TABLE 2: EFFECT OF *ECLIPTA ALBA* ON DOPAMINE AND 5HT LEVELS IN CU STRESS INDUCED NEUROTRANSMITTER ALTERATION IN DISCRETE REGION OF BRAIN

Groups	Dopamine			5HT		
	PFC	CS	HI	PFC	CS	HI
Control	785_16 ± 1487840	6515_50 ± 46_68	1524_163 ± 26_82	771_83 ± 3_18	5732_167 ± 71_17	3704_33 ± 10_32
AUS	2627_66 ± 89_17417*$	4601_77 ± 84_61*	2748_3333 ± 53_00*	795 ± 4_96*$	10841_3 ± 134_18*$	3977_666 ± 1_211*$
AUS + EA	2096_0 ± 39_93*@	8539_16 ± 31_61*@	235_16667 ± 73_36*@	782 ± 5_03*	8208_27 ± 768_89*	3615_66 ± 14_43*@
CUS	2748_5 ± 26_69*	9205_5 ± 15_78*	32068_3 ± 15_63*	734_16 ± 2_33*	5194_3 ± 157_04*	3768_16 ± 12_36*
CUS + EA	1261_0 ± 10_71#	7872_83 ± 8_010*#	2345_33 ± 22_68*#	767_66 ± 6_67*#	6660_83 ± 18_26*#	3655_83 ± 22_22*#
EA	765_83 ± 14_06	6565_83 ± 8_113	1548_66 ± 19_66*	768_66 ± 3_82	5692 ± 143_80	3787 ± 41_8

Data are expressed as mean ± SD. Significance set at $p < 0.05$, *- compared with control, #- CUS compared with CUS+EA, @- AUS compared with AUS+EA, $- CUS compared with AUS

Biphasic Response of 5HT Table 2: Levels with stress is observed, where acute stress in the hippocampus (df-5, F-218.98), Corpus striatum (df 5, F-257.513), PFC (df 5, F-58.362) were invariably increased. Whereas in Chronic unpredictable stress group levels were significantly decreased as compared with control ($p < 0.05$). Indicating difference in altered neurotransmitter levels in response to stress; respective treatment group showed decrease from that of stress group though was not normalized to control. EEEA treated group did not deviate from that of control.

DISCUSSION: Stress is experienced during situations that pose a threat to an organism and leads to the activation of two major physiological systems, the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenal (HPA) axis. Activation of the HPA axis results in release of corticosteroids from the adrenal cortex [30, 31] in our study Corticosterone levels were found to be high both in acute as well as chronic unpredictable stress. Studies have reported that Corticosteroid released as a result of chronic stress has adverse effect on health [32] whereas acute rise in the corticosterone levels have shown to be beneficial with regard to cognition by improving learning and memory hippocampus which is crucial for formation of declarative memory and spatial memories and prefrontal cortex responsible for working memory and higher order cognitive function have a high density glucocorticoid receptors which makes them highly susceptible for adverse effects of stress.

After 30 days of the chronic unpredictable stress, the rats showed decreased recognition memory and altered innate behavior of novel object exploration. Absolute novel exploratory time was less in CUS group animals as compared to control. Acute stress did not show much deviation from control. These behavioral changes were paralleled by biochemical alterations, including higher plasma levels of corticosterone. The present results showed the modulatory effects of *Eclipta alba* Linn. Administration to improve the serum corticosterone level in the rats submitted to chronic unpredictable stress. The chronic stress exposure can disturb the oxidant and antioxidant balance, and induce a large production of free radicals and suppress antioxidant capacity.

Neuronal biochemical composition is mainly susceptible to ROS since it involves plenty of unsaturated lipids labile to peroxidation and oxidative modification. Free radicals destruct double bonds of unsaturated fatty acids and that initiates cascade of reaction to damage neighboring unsaturated fatty acids [33]. Brain consumes an inordinate fraction (20%) of total oxygen which makes it more susceptible for peroxidation[34]; also it is not particularly enriched in antioxidant defenses.

Brain is lower in antioxidant when compared to other organs, it is around 10% of liver [35]. Oxidative stress is characterized by reduced antioxidant defense components, such as catalase, glutathione, total thiol *etc.,* or elevated levels of ROS and RNS in the body [36]. In this study antioxidants superoxide dismutase, catalase, gpx, glutathione reductase, Vitamin C, Vitamin E were significantly reduced in the chronic stress group whereas was significantly increased in acute stress group in discrete brain regions as rise in antioxidants are the first line

defense for acute increase in free radicals generated, our findings are supported in a study in which rise in catalase and SOD are observed after acute restrainer stress [37]. *Eclipta alba* is a rich natural source of antioxidant and thereby abolished oxidative stress. It was observed that *Eclipta alba* treatment significantly reduced TBARS, H_2O_2 and nitric oxide levels and restored antioxidant defense.

SNS activation allows for the immediate fight-or-flight response through rapid release of epinephrine (EPI) and norepinephrine (NE) from the adrenal medulla [38] Under normal conditions, NE is a principle component of the stress response, directly increasing heart rate and blood flow to skeletal muscles and triggering the release of glucose, all in preparation for the 'fight-or-flight' response. Persistent noradrenergic activity however has negative impact on cognition. In our study acute and chronic stress induced opposed response in 5HT levels in different brain regions. Where acute stress showed increase and chronic stress showed decrease in the 5HT levels. As Lower 5-HT has also been implicated in diminished physical health [39]. Cognitive declaimed cognition can be linked to lower 5HT levels in chronic unpredictable stress group animals.

Dopamine (DA) is a neurotransmitter that plays a major role in emotion and the reward system of the brain. It optimally functions within a narrow range and dopaminergic hypo- or hyperactivity is implicated in both physical and psychiatric illnesses. Parkinson's disease is characterized by a loss of dopaminergic neurons, and evidence suggests schizophrenia and psychosis are linked to elevated levels of DA [40]. Based on the previous research, it is clear that elucidating the complex interactions of the numerous neurotransmitter systems may be a critical link in understanding cognition impairment progression. Altered neurotransmitters are normalized in this study by treatment with ethanolic extract of *eclipta alba* Linn. Previously in many studies herbs have been used for combating stress induced alteration in the neurotransmitter [41, 42, 43]. Stressful events have profound effects on learning and memory.

Extensive evidence from animal and human studies indicates that stress and glucocorticoids influence cognitive function. There is evidence showing that male rats, but not female, show impaired performance in the NOR task after 21 days of chronic stress. These results are also reflected at the neural and endocrine levels, where male rats show significant atrophy of apical dendritic branches of the CA3c pyramidal neurons [44]. Further, Electrophoresis of DNA isolated from whole brain showed smear pattern **Fig. 6**.

This result shows that chronic unpredictable stress induces DNA damage in the brain. This result is supported by a study in which [45, 46] oxidative DNA damage is associated with intense noise exposure in the rat. DNA can be an early target of damage in mammals, as strand breakage occurs before detectable lipid peroxidation or protein damage. Further *Eclipta alba* Linn. treatment in the stressed animals showed significantly reduced DNA damage.

CONCLUSION: Present study confirms the previous findings that CUS alters neuro-transmission and antioxidant defense system in different regions of brain and also causing disruption at DNA level there by impairing cognition. *Eclipta alba* Linn. a dietary herb possesses neuroprotective actions that has contributed to the purported enhancement of cognitive function.

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