Research Article

Attenuating Effect of *Elsholtzia Communis* (Collett & Hemsl.) Diels. on Dysregulated HPA Axis in Stressful Conditions

Barua C C[1*], Patowary P[1], Bordoloi M J[2], Dutta P P[2], Dutta D J[2], Purkayastha A[1], Barua I C[3]

[1]*Department of Pharmacology and Toxicology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati.*
[2]*Natural Products Chemistry Division, CSIR-Northeast Institute of Science and Technology, Jorhat, Assam.*
[3]*Department of Agronomy, Assam Agricultural University, Jorhat, Assam*

ABSTRACT

The study was conducted to evaluate the adaptogenic activity of hydroethanolic extract of *Elsholtzia communis* (Collett & Hemsl.) Diels. by estimation of neurobiological and biochemical changes associated with acute and chronic stress. Adult male Wistar rats were given forced swimming induced acute and chronic stress for 3 and 7 days, respectively. Hydroethanolic extract of *Elsholtzia communis* (EC) was orally administered at 100 (EC-100) and 200 (EC-200) mg/kg doses. For comparison, Panax ginseng (PG) extract (100 mg/kg, p.o.) was used as standard adaptogen. Plasma corticosterone, BDNF level, brain levels of lipid peroxides (LPO), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), cortical and hippocampal levels of monoamines viz. norepinephrine (NE), epinephrine (Ep) and dopamine (DA) were quantified for ascertaining biochemical changes accompanying stress. The results were further confirmed by the estimation of various proinflammatory cytokines (IL-6, IL-1β and TNF-α) in serum. As compared to vehicle treated stressed rats, the EC and PG treated significantly normalised chronic acute and chronic stress induced elevation of corticosterone and reduction of plasma BDNF; both doses (EC-100 and EC-200) stabilized the irregular oxidative status of the brain in stressed rats. EC treatment further suppressed the elevated levels of IL-6, IL-1β and TNF-α in stressed animals. The leaf of the plant is consumed by the local tribes in Nagaland, Arunachal Pradesh of northeast India, easily cultivated by farmers for its immense popularity. Hence, our study as an adpatogen has added one more medicinal property of this food plant, which can be successfully utilised as an adpatogen to combat adverse climatic condition prevailing in those areas.

Key words:

INTRODUCTION

Stress is basically a process that contributes to the retrieval of homeostasis and as a result of which the brain-body integration most evidently occurs[1,2]. Augmented levels of circulating glucocorticoids that ensues after the instigation of the hypothalamic–pituitary–adrenal (HPA) axis are probably the central mediator in this response[3]. The relationships amid brain-derived neurotrophic factor (BDNF), stress, and depression have been reported in numerous animal studies[4-6]. It was also reported that forced swimming decreases BDNF mRNA in the hippocampus[7]. The difference between serum and plasma levels of BDNF could reflect the amount of BDNF stored in circulating platelets. Circulating BDNF might originate from neurons and glial cells of the brain, since BDNF is known to cross the blood-brain barrier in both directions[8,9]. Therefore, plasma BDNF may reflect circulating levels rather than that stored in platelets. Furthermore, in experimental animals, it has been shown that psychological stressors leads to upsurge of pro inflammatory cytokine levels such as IL 1β, IL-2, IL-6 and TNF-α in blood and in various brain regions[11,12]. Moreover, brain is known to be susceptible for oxidative damage because of its high utilization of oxygen[13]. There are several studies that are related to the effects of stress on the antioxidant system and induction of lipid peroxidation in brain after various stress exposure models[14]. Again, out of various neurotransmitters, norepinephrine (NE), epinephrine (Ep), dopamine (DA) and 5-hydroxy tryptamine (5-HT) are the important monoamines well established during stressful conditions[15]. Since the introduction of adaptogen conception[16] numerous plants have been investigated, which were used earlier as tonics due to their adaptogenic and rejuvenating properties in traditional medicine[17,18]. *Elsholtzia communis* (Collett & Hemsl.) Diels, (Lamiaceae)[19], have been widely distributed and used in East Asia, Africa, North America, and European countries for centuries. *Elsholtzia* is reported to cover the activities of antiviral, antibacterial, anti-inflammatory, anti-oxidant, and myocardial ischemia protection, as well as other activities[17]. The leaves of the plant is consumed by many people of north eastern states and is popularly as
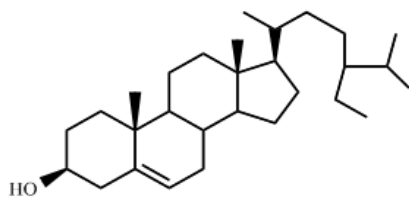
Figure 1: Chemical structure of the isolated pure compound of EC

a spice, herbal tea, cosmetics, perfumery and condiment presumably due to its effect on digestion. Proximate composition and mineral contents of the leaves showed high protein content[20]. But its anti stress or adaptogenic study so far has not been evaluated. There are several studies that are related to the effects of stress on the antioxidant system and induction of lipid peroxidation in the brain after various stress exposure models[21,22] pertaining to many plants or herbs, which is lacking in *Elsholtzia communis* (EC). With this background, the present work has been undertaken to assess the adaptogenic potency of EC.

MATERIALS AND METHODS

Experimental Animals
Adult male Wistar rats weighing between 170-200 g were used for the experiment. One day prior to the experiment, the animals were allowed for acclimatization to the condition overnight. Rats were housed three to four per cage at a constant temperature (22 ± 2 °C) and 12/12 h light/dark cycle and were fed standard laboratory food, water was given *ad libitum*. Effort was made to minimize suffering of the experimental animals throughout the study.

Chemicals
Ginseng, Folin Ciocalteu reagent, cacodylic acid, n-nitrobluetetrazolium, triton-X, pyrogallol, metaphosphoric acid, EDTA, GSH standard, thiobarbituric acid, 5,5-dithiobis (2-nitrobenzoic acid), dopamine, norepinephrine, epinephrine, heptane sulphonic acid were used for estimation of various parameters. All chemicals were of Sigma, St. Louis, MO, USA grade. Standards were prepared at 1 mg/ml in 0.2 N Perchloric Acid (PCA) and other chemicals all of high purity/HPLC grade were used.

Plant Materials
Fresh leaves of the plants were collected from the local market of Dimapur, Nagaland. Herbaria of the plant was prepared by taxonomist Dr. I.C Barua and authenticated from Central National Herbarium, BSI, Howrah, West Bengal. Voucher specimen (Barcode no. CAL 0000027003) was deposited at AAU, Khanapara.

Preparation of Extract and Isolation of Compound
Based on our previous studies, hydroethanolic extract of EC was found to possess better adaptogenic potential compared to other extracts. Therefore, hydroethanolic extract of the leaves of EC was selected for the present study. Briefly, about 250 g of powdered material was soaked in a mixture of 1000 ml ethanol and water (1:1) for 72 h in a beaker and mixture was stirred every 18 h

using a sterile glass rod. Filtrate was obtained three times with the help of Whatman filter paper no. 1 and the solvent was removed by rotary evaporator (Buchi R-210, BÜCHI Labortechnik AG, Meierseggstrasse Switzerland) under reduced pressure at < 45 °C temperature leaving a dark brown residue. It was stored in airtight container at 4 °C until use. The recovery percentage was found to be 18.63 % w/v. Based on acute toxicity studies (OECD guidelines no. 423), two doses *viz.* 100 and 200 mg/kg p.o. (EC–100 and EC–200) were selected for the study. The hydroethanolic extract of EC was again fractionated with hexane followed by chloroform. Chloroform fraction of hydroethanolic extract of EC was chromatographed over a column of silica gel using solvent system consisting of hexane and ethyl acetate mixture of increasing polarity.

General Experimental Procedures for the Isolation of Compound
IR spectra were recorded on a Perkin Elmer System2000 FTIR spectrometer. ^{1}HNMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AVANCE DPX 300 NMR spectrometer in CDCl$_3$ using TMS as internal standard and mass spectra were recorded on Mass spectrometer, Trace DSQ GCMS (M/S Thermo Fisher Scientific) system. Silica gel G was used for TLC. Flash chromatography of extracts was done on Teledyne combi flash system. All solvents used were distilled prior to use. Ethanol extract of EC was fractionated with hexane followed by chloroform three consecutive times. Hexane, chloroform and remaining fractions were evaporated to dryness. Chloroform fraction of ethanol extract of EC (30 g) was chromatographed over a column of 600 g of silica gel (60-120 mesh) starting with the solvent system hexane and 200 ml fractions were collected. The collected fractions were analyzed by TLC in different solvent systems. Similar fractions were combined and were evaporated under reduced pressure at 45 °C using rotary evaporator. Combined fractions of 1-5 (1:2, ethyl acetate: hexane) and fractions 6-9 (1:1, ethyl acetate: hexane), was again subjected to preparative TLC using the solvent system ethyl acetate: hexane (1:2).

Induction of Stress[23]
Among the numerous methods employed, water immersion stress i.e. forced swimming test was used as it is considered to be one of the best model of stress which provides both emotional stress (despair behavior) as well as physiological (vigorous muscular activity) stress to the animal and has been used extensively and accepted widely.

Experimental Design
All the compounds were dissolved in vehicle comprising of distilled water and tween 80 (0.1 %) solution. Drugs were prepared fresh daily before administration. All experiments were performed according to current guidelines for the care of laboratory animals which was approved by IAEC (No.773/ac/CPCSEA/FVSc,AAU/IAEC/10-11/72).

Animals were randomized in 5 groups (n = 6) each for acute stress (AS) and chronic stress (CS). The groups and the treatments were as follows:
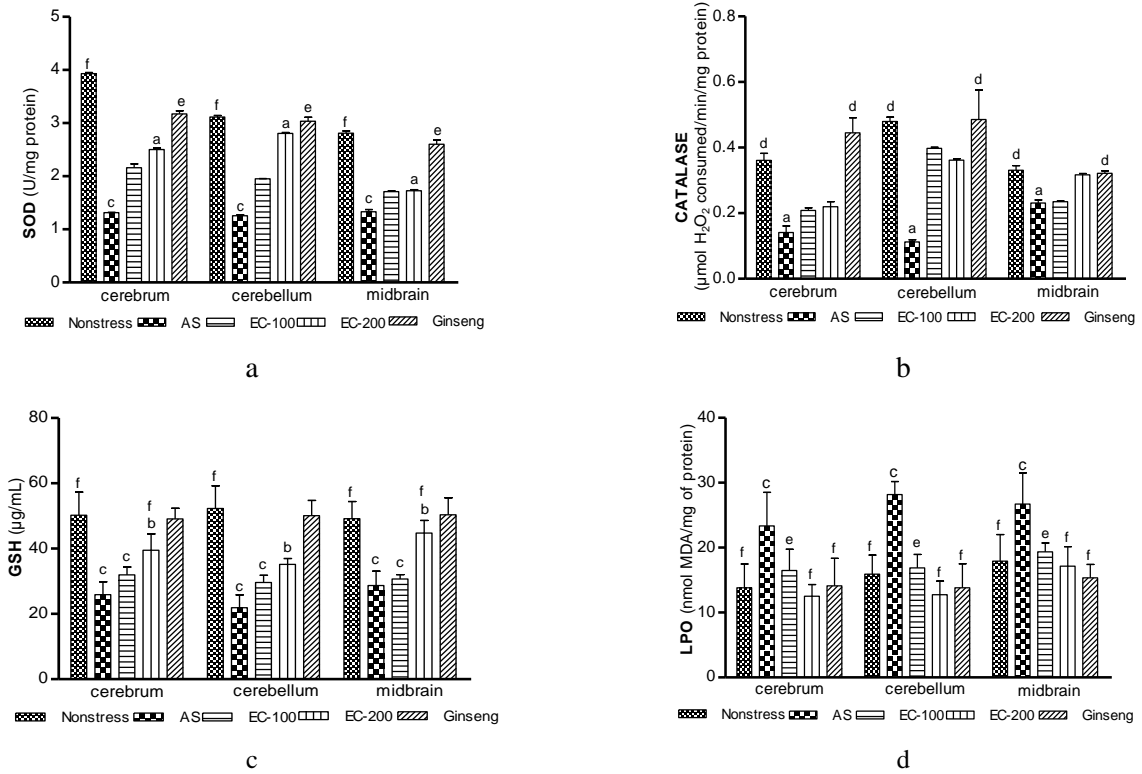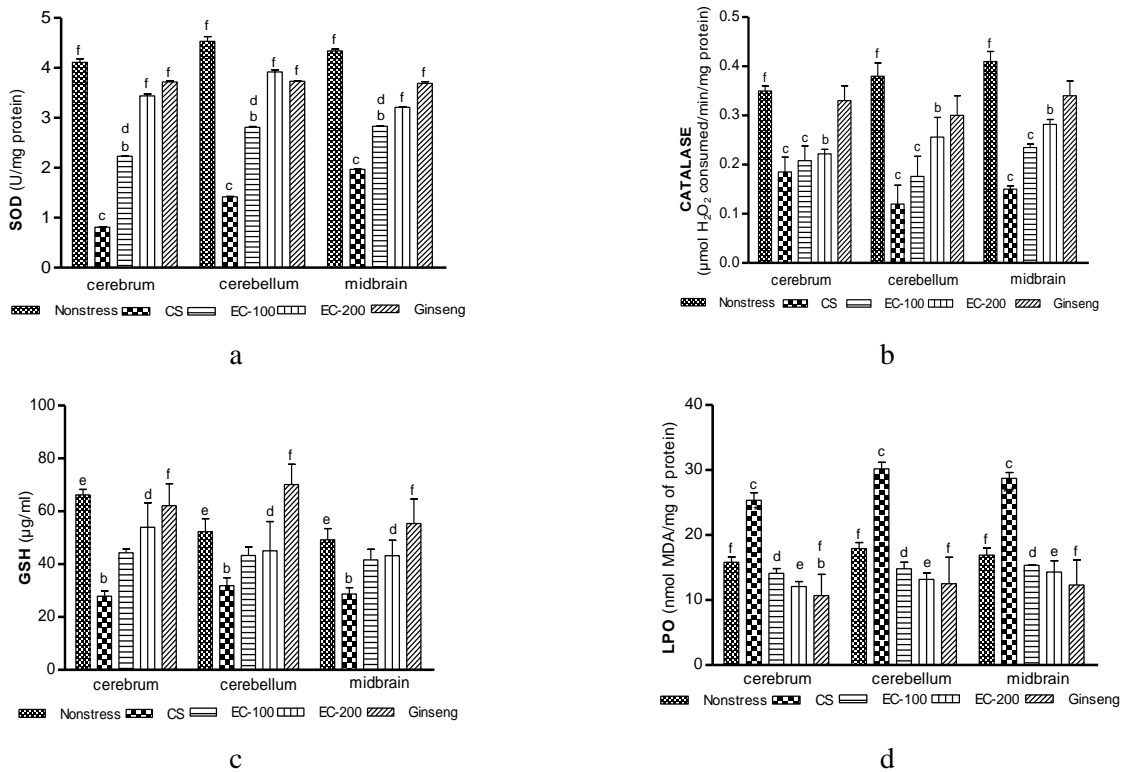
a

b

c

d

Figure 2a-2d: Graphical representation of the effect of EC-100 and EC-200 on brain antioxidant level following AS. All values are expressed as mean ± SEM (n = 6). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, vs. non stress and [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ vs. AS induced group

a

b

c

d

Figure 3a-3d: Graphical representation of the effect of EC-100 and EC-200 on brain antioxidant level following AS. All values are expressed as mean ± SEM (n = 6). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, vs. non stress and [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ vs. AS induced group

Group I – Vehicle treated (Non stress)   Group III - EC-100 treated + Stress
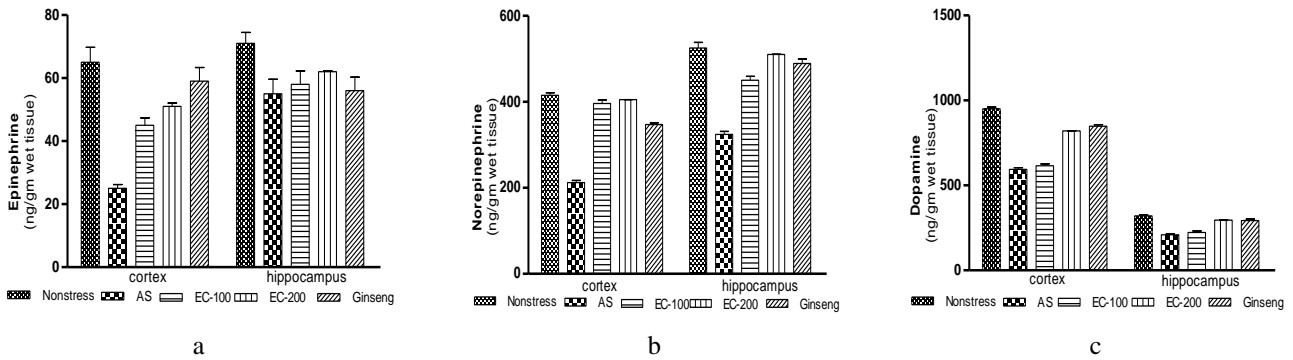Group II - Vehicle treated + Stress   Group IV - EC-200 treated + Stress

Figure 4a-c: Graphical representation of the effect of EC-100 and EC-200 on cortical and hippocampal catecholamine level following AS. All values are expressed as mean ± SEM (n = 6).[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, , vs. non stress and [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ vs. AS induced group.
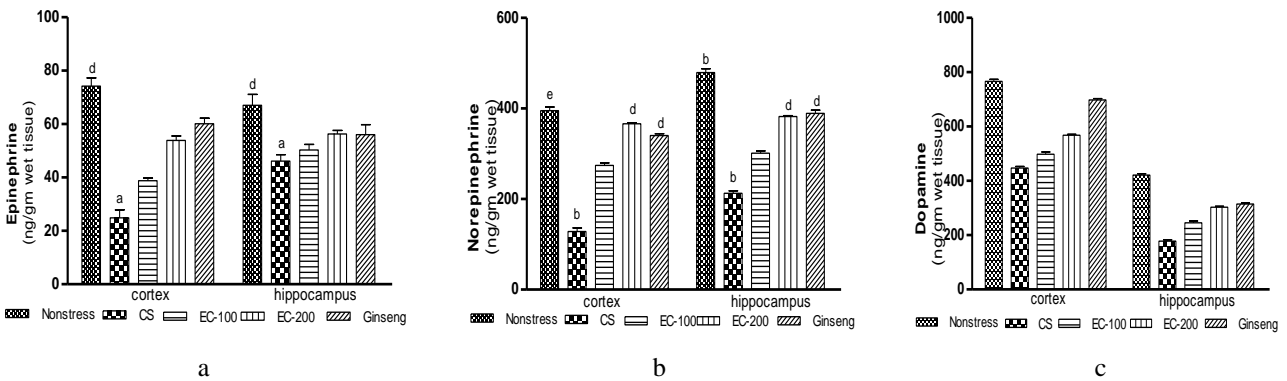

Figure 5a-5c: Graphical representation of the effect of EC-100 and EC-200 on cortical and hippocampal catecholamine level following AS. All values are expressed as mean ± SEM (n = 6).[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, , vs. non stress and [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ vs. AS induced group.

Group V - Ginseng (100 mg/kg p.o.) + Stress

The AS drug groups were fed with EC-100 and EC-200 p.o. daily for 3 days. A similar group of rats were fed with vehicle (10 ml/kg p.o.) for the same number of treatment days but were not subjected to stress, in order to obtain standard data for different parameters. On the second day, after feeding drug or vehicle, animals were fasted overnight with free access to water. On the third day, 45 min after feeding the drug or vehicle, rats were given stress by forced swimming for 20 min except the non stress group. In CS, the drugs were fed daily 45 min prior to stress regimen, up to seven (7) consecutive days except that the rats were fasted overnight on the sixth day after completion of the experimental regimens of drug feeding and stress exposure. A parallel non stress control group was also taken as described above and euthanized on seventh day along with the CS group of rats. The animals were given mild anesthesia using diethyl ether and blood was collected. Later on, the animals were sacrificed humanely and whole brains were excised and washed in cool saline solution (0.9 %). Subsequently, cerebrum, cerebellum and midbrain areas were dissected out, blotted using filter paper and weighed.

Enzyme Assays

Homogenization of the brain was carried out at 4 °C and centrifuged at 10000 g for 15 min. The supernatant was collected and taken for estimation of protein[24], lipid peroxidase (LPO)[25], superoxide dismutase (SOD)[26],

catalase[27] and reduced glutathione (GSH)[28] with slight modification.

Estimation of Catecholamines

Norepinephrine (NE), epinephrine (Ep) and dopamine (DA) concentrations were estimated in the brain prefrontal cortex and hippocampus with slight modification of the method by Sheikh et al. 2007[29]. An Ultimate 3000 HPLC system (U-HPLC), Thermo Scientific Dionex Coulochem III electrochemical detector, Acclaim® 120 C18 Column 4.6 x 250 mm 5 µm (Thermo Scientific Dionex, USA) was used for the estimation of catecholamines and corticosterone. An electrochemical detector (5041A) consisting of a glassy carbon electrode versus an Ag/AgCl reference electrode maintained at a working potential of -150 mV (E1) and +250 mV (E2). The concentrations of catecholamines were calculated using a standard curve generated by determining the ratio between three samples of known amounts and with the internal standard in triplicate.

Estimation of Plasma Corticosterone

The collected blood was kept in ice and centrifuged at 10000 g for 20 min at 4 °C. Plasma was separated and aliquots were stored at –80 °C for corticosterone estimation. An HPLC/UV system (UHPLC, Dionex) was used for quantification of plasma corticosterone by the method of Woodward and Emery, 1987[30] with minor modifications.

Table 1a: Effect of EC-100 andEC-200 on plasma corticosterone and BDNF level following AS. All values are expressed as mean ± SEM, (n = 6). [a]p < 0.05, [b]p < 0.01, [c]p < 0.001, , vs. non stress and [d]p < 0.05, [e]p < 0.01, [f]p < 0.001 vs. AS induced group.

Treatment	Corticosterone (ng/mL)	BDNF (pg/mL)
Non stress	323.93 ± 0.081[d]	213.564 ± 5.470[e]
Acute Stress	408.79 ± 0.048[a]	118.672 ± 0.112[c]
EC-100	265.23 ± 0.091	159.370 ± 5.534[ad]
EC-200	279.39 ± 0.162[d]	158.030 ± 5.113[ae]
Ginseng	271.81 ± 0.134	259.066 ± 6.822

Table 1b: Effect of EC-100 and EC-200 on plasma corticosterone and BDNF level following CS. All values are expressed as mean ± SEM, (n = 6). [a]p < 0.05, [b]p < 0.01, [c]p < 0.001, , vs. non stress and [d]p < 0.05, [e]p < 0.01, [f]p < 0.001 vs. CS induced group

Treatment	Corticosterone (ng/mL)	BDNF (pg/mL)
Non stress	351.654 ± 3.092[e]	229.8733 ± 10.838[d]
Chronic Stress	518.143 ± 2.073[b]	92.883 ± 6.449[a]
EC-100	282 ± 0.097[bd]	156.615 ± 0.835[b]
EC-200	258 ± 0.105[c]	102.773 ± 9.972[a]
Ginseng	292.675 ± 2.189[ad]	263.641 ± 7.700[ad]

Estimation of Plasma BDNF and Serum Cytokines by ELISA

Plasma BDNF, serum IL–1β, IL–6, TNF–α level was assayed by an enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen Corpomiceion, Camarillo, CA). The detection limit of the assay was 12 pg/ml for BDNF and 5 pg/ml for cytokines.

Statistical Analysis

Results are presented as Mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) among different treatment groups followed by Tukey–Kramer multiple comparison test (Graph Pad Prism 5.0). p < 0.05 was considered to indicate statistical significance.

RESULTS

Isolation of the Pure Compound

The repeated chromatography of fraction of ethyl acetate and hexane 1:7 solvent system afforded a pure compound. The ^{1}HNMR, ^{13}CNMR, IR and MS spectra were recorded for the pure compound. The study of these spectroscopic data allowed identifying the compound as Figure 1. The percentage yield of the compound was found to be 0.016 % and melting point was recorded at 140 ºC.

Oxidative Enzymes
SOD, CAT and GSH

Following acute and chronic stress regimen in the experimental animals, elicited decrease in brain antioxidant levels *viz.* SOD, CAT and GSH. In the cerebrum, significant (p < 0.05) decrease in SOD (1.313 ± 0.007 and 0.81 ± 0.009 U/mg protein), CAT (0.141 ± 0.020 and 0.185 ± 0.030 µmol H_2O_2 consumed/min/mg protein) and GSH (31.95 ± 2.40 and 27.88 ± 1.98 µg/ml) was observed in comparison to non-stress control groups (3.93 ± 0.02 4.11 ± 0.07 U/mg protein for SOD, 0.36 ± 0.02 and 0.35 ± 0.01 µmol H_2O_2 consumed/min/mg protein for CAT, and 50.21 ± 7.09 and 66.21 ± 2.09 µg/ml for GSH). Similar trend was observed in cerebellum and midbrain also. The results are depicted in Figure 2a to 2c and Figure 3a to 3c. The levels of GSH, CAT and SOD were significantly (p < 0.05) elevated in different regions (cerebrum, cerebellum and midbrain) of the brain following treatment with EC-200.

LPO

Acute and chronic exposure to stress stimulated a significant increase (p < 0.05) in LPO as indicated by the accumulation of malondialdehyde (MDA) in brain tissue of animals, presented in Figure 2d and 3d. The results are analogous in cerebrum, cerebellum and midbrain as well. Pretreatment with EC significantly (p < 0.05) affected the activity of LPO enzymes which in turn combat harmful effect of free radicals in acute and chronic stress induced rats. The effect was comparable with that of standard drug ginseng.

Catecholamines

Both AS and CS affect the catecholamine levels in the cortex and hippocampus region of the brain of rats. Depletion of NE stores was observed in acute and chronic stress in the cortex (212.00 ± 5.22 and 128.20 ± 8.20 ng/gm wet tissue) and hippocampus (324.60 ± 7.04 and 212.79 ± 5.67 ng/gm wet tissue) as compared to non-stress control groups for AS (415.17 ± 6.12 in cortex and 525.03 ± 13.03 ng/gm wet tissue in hippocampus) and CS (395.20 ± 7.93 in cortex and 479.11 ± 8.50 ng/gm wet tissue in hippocampus). Treatment with EC-200 (p < 0.05) significantly reverted decreased NE levels following AS (396.20 ± 7.12 in cortex and 450.45 ± 8.30 ng/gm wet tissue in hippocampus) and CS (366.21 ± 2.01 ng/gm wet tissue in cortex and 382 ± 2.33 ng/gm in hippocampus) more effectively than 100 mg/kg oral dose. Similar trend was observed in the levels of cortical and hippocampal Ep and DA. However, pretreatment with EC-100 and 200 countered the deleterious effect of AS and CS resulting in significant (p < 0.05) elevation in the levels of Ep and DA after AS and CS. The attenuation of the levels of catecholamine after treatment with EC-200 was better than that of EC-100 and was similar to that of ginseng. Results are depicted in Figure 4a-4c and Figure 5a-5c.

Plasma Corticosterone and BDNF

The HPLC results have shown that in the non-stress group for AS and CS, the corticosterone level was 323.93 ± 0.081 and 351.654 ± 3.092 ng/mL respectively (Table 1a and 1b). Whereas, following stress, it was elevated to

Table 2a: Effect of EC-100 and EC-200 on serum cytokine levels following AS. All values are expressed as mean ± SEM, (n= 6).[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, , vs. non stress and [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ vs. AS induced group.

Treatment	IL 1β (pg/mL)	IL 6 (pg/mL)	TNF α (pg/mL)
Non stress	671.020 ± 2.930[d]	224.671 ± 1.796[d]	895.008 ± 2.143[d]
Acute Stress	836.171 ± 2.396[a]	432.561 ± 2.621[b]	1330.512 ± 4.175[b]
EC-100	732.500 ± 3.174	384.032 ± 3.410	1275.620 ± 4.991[a]
EC-200	697.912 ± 3.021[bd]	321.720 ± 3.771	1081.831 ± 5.190[ae]
Ginseng	690.225 ± 2.130[a]	290.301 ± 3.406[b]	948.292 ± 4.761

Table 2b: Effect of EC-100 and EC-200 on serum cytokines level following CS. All values are expressed as mean ± SEM, (n = 6). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, , vs. non stress and [d]$p <0.05$, [e]$p < 0.01$, [f]$p <0.001$ vs. CS induced group.

Treatment	IL 1β (pg/mL)	IL 6 (pg/mL)	TNF α (pg/mL)
Non stress	650.343 ± 3.191[d]	247.012 ± 3.174[d]	781.212 ± 3.226[d]
Chronic Stress	1140.127 ± 5.112[a]	571.657± 5.871[b]	1367.354 ± 3.400[b]
EC-100	901.565 ± 4.921	454.293 ± 4.140	1190.981 ± 3.672[b]
EC-200	845.231 ± 4.732[bd]	389.609 ± 3.331	1023.657 ± 4.526[be]
Ginseng	766.440 ± 5.089[a]	305.382 ± 4.165[b]	912.201 ± 3.779

408.79 ± 0.048 and 518.143 ± 2.073ng/mL in AS and CS groups, respectively. Treatment with EC-100 resulted in significant (p < 0.01) lowering of the plasma corticosterone to near normal level after CS (282 ± 0.097 ng/mL), and non-significantly to 265.23 ± 0.091 ng/mL after AS. Furthermore, better effects were observed with EC-200 after AS (279.39 ± 0.162 ng/mL) and CS (258 ± 0.105 ng/mL), which was comparable with the standard drug. Following stress, plasma BDNF level was reduced to 118.672 ± 0.112 and 92.883 ± 6.449 pg/mL in AS and CS groups, respectively. Significant elevation was observed following treatment with EC-100 and EC-200 to standard level subsequent to AS (159.370 ± 5.534 and 158.030 ± 5.113 pg/mL), and CS (156.615 ± 0.835 and 102.773 ± 9.972 pg/mL) which was comparable with ginseng.

Serum Cytokines

Exposure to AS and CS has significantly (p<0.01) and obviously increased proinflammatory cytokines IL-1β (836.171 ± 2.396 and 1140.127 ± 5.112 pg/mL), IL-6 (432.561 ± 2.621 and 571.657 ± 5.871 pg/mL) and TNF-α (1330.512 ± 4.175 and 1367.354 ± 3.400 pg/mL) levels as compared to the non stress control groups. Pretreatment with EC-100 significantly (p < 0.05) reduced AS induced elevation in IL-1β, IL-6 and TNF-α level. The decrease in the levels of these stress induced proinflammatory cytokines was better with the EC-200 treated groups (697.912 ± 3.021 pg/mL IL-1β, 321.720 ± 3.771 IL-6 and 1081.831 ± 5.190 TNF-α for AS; 845.231 ± 4.732 pg/mL IL-1β, 389.609 ± 3.331 IL-6 pg/mL and 1023.657 ± 4.526 pg/mL TNF- α for CS) which was comparable with ginseng. The results are presented in Table 2a and 2b.

DISCUSSION

In the pursuit of new therapeutic products for the treatment of neurological disorders, medicinal plant research, globally, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. In the present study, the antistress activity of the hydroethanolic extract of EC (100 mg/kg and 200 mg/kg)

has been evaluated using acute and chronic stress in experimental models. The forced swimming test is a known physical stress model or the evaluation of antistress activity. The immobility displayed by rodents when subjected to unavoidable stress such as forced swimming, is thought to reflect a state of despair or lowered mood[31]. Exposure to stress leads to characteristic depression of the free radical defense mechanism including alterations in the activities of antioxidant enzymes, exposing the body's own antioxidant defense system to damage by ROS[32]. Our study revealed that elevated levels of LPO and the lesser levels of SOD, CAT and GSH in acute and chronically stressed rats were all reversed by EC treatments. These observations might indicate that the conjugates present in the extract could as well be involved in these effects of EC. The present data showed that forced swimming stress led to a significant depletion of cortical and hippocampal catecholamine stores. A variety of stressors induce a significant alteration in the metabolism and function of various neurotransmitters in the CNS as well as peripheral nervous system. In AS, the effect of the test compound was found to elicit a better response in normalizing the brain catecholamine stores but the effect was not long term. Again, at higher dose i.e. 200 mg/kg, p.o. treatment was found to prevent the stress induced depletion of NE, Ep and DA much more effectively during CS thus helping the organism to cope up better during stress. Previous studies reported that stressful conditions lead to significantly decreased level of monoamines (NE, Ep and DA) in all the brain regions and plants *viz. Bacopa monniera*,[33], *Annona muricata* and *Polyalthia cerasoides*[34] reverted the changes back to normal. Our study is in agreement with the above reports that these changes (decrease in NE, Ep and DA) in AS as well as CS tend to return to normal levels with EC-100 and EC-200 treatment. Again, in response to stress, the adrenocorticotropic hormone (ACTH) is released, which acts on the adrenal cortex to stimulate the synthesis and release of cortisol[35]. Increase in plasma cortisol influences the mobilization of stored fat and carbohydrate reserves,[36] which in turn increases the blood glucose

level, total proteins, cholesterol, and triglycerides. We have also reported in our previous studies that pretreatment with EC-100 and 200 significantly ameliorated the stress-induced variations in these biochemical levels but returned to normal following treatment with the same extract. Several studies utilizing micro dialysis techniques have shown an increase in the levels of corticosterone in prefrontal cortex, hippocampus and amygdala during an acute stressful challenge[34]. Subsequently, we estimated levels of pro-inflammatory cytokines IL-6, IL-1β TNF- α and IL-1β in plasma of vehicle or EC treated stressed animals. Results of the experiments revealed that pro-inflammatory cytokines IL-1β, IL-6 and TNF-α expressions in the vehicle treated stressed rats were much higher than those of a vehicle treated unstressed one, and that the stress-induced effects were completely suppressed by the higher dose (200 mg/kg) of EC. The efficacy of most herbal remedies is attributed to various active principles, in combination. Results of phytochemical screening showed the presence of polyphenolic compounds like flavonoids, triterpenes and steroids in the leaves. It is therefore probable that the components that are present in abundance in the extracts might contribute in part to the observed antistress effect. In conclusion, our results provide evidence that the seven-day treatment with the hydroethanolic extract of EC leaves shows antistress (adaptogenic) activity in stress models in a better way than the three-day treatment. The observed antistress activity may be due to the prevention of desensitization of both the peripheral and central components of HPA axis and due to the non-specifically increased resistance produced by the EC extract. All these effects indicate the attenuating effect of *Elsholtzia communis* on dysregulated HPA axis in stressful conditions. Since the plant is popularly used by the natives in various states due to their food value or medicinal property, adaptagenic activity can be easily exploited for the welfare of animals and human inhabiting in the remote areas with adverse climatic conditions like extreme heat or cold.

REFERENCES
1. de Kloet ER, Joëls M and Holsboer F. Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* (2005) 6:463-75.
2. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* (2007) 87:873-904.
3. Armario A. The hypothalamic–pituitary–adrenal axis: what can it tell us about stressors? CNS *Neurol. Disord. Drug Targets* (2006) 5:485-501.
4. Altar CA, Whitehead RE, Chen R, Wortwein G, Madsen TM. Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. *Biol. Psychiatry* (2003) 54:703-9.
5. Duman RS and Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol. *Psychiatry* (2006) 59:1116-27.
6. Russo-Neustadt AA, Alejandre H, Garcia C, Ivy AS and Chen MJ. Hippocampal brain-derived neurotrophic factor expression following treatment with reboxetine, citalopram, and physical exercise. *Neuropsychopharmacol.* (2004) 29:2189-99.
7. Russo-Neustadt A, Ha T, Ramirez R and Kesslak JP. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. *Behav. Brain Res.* (2001) 120:87-95.
8. Pan W, Banks WA, Fasold MB, Bluth J and Kastin AJ. Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacol.* (1998) 37:1553-61.
9. Poduslo JF and Curran GL. Permeability at the blood–brain and blood–nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. *Mol. Brain Res.* (1996) 36:280-86.
10. Ishikawa I, Kitamura H and Kimura K. Brain interleukin-1 is involved in blood interleukin-6 response to immobilization stress in rats. *Jap. J. Vet. Res.* (2001) 49:19–25.
11. Nguyen KT, Deak T and Owens SM. Exposure to acute stress induces brain interleukin-1beta protein in the rat. *J Neurosci* (1998) 18:2239-46.
12. Hyang DG, Kwang HL, Hyoung JK, Eun HL, Jiyong L and Youn SS. Neuroprotective effects of antioxidative flavonoids, quercetin, dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica* var saboten. *Brain Res.* (2003) 965:130-6.
13. Madrigal JL, Olivenza R, Moro MA, Lizasoain I, Lorenzo P and Rodrigo J. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. *Neuropsychopharmacol.* (2001) 24:420-9.
14. Tsigos C and Chrousos PG. Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *J. Psychosom. Res.* (2002) 53:865-71.
15. Gonzalo A, Carrasco LD and Van DK. Neuroendocrine pharmacology of stress. *Eur. J. Pharmacol.* (2003) 463:235-72.
16. Rege NN, Thatte UM and Dahanukar SA. Adaptogenic properties of six rasayanas herbs used in ayurvedic medicine. *Phytother. Res.* (1999) 13:275-91.
17. Das A, Shanker G, Nath C, Pal R, Singh S and Singh H. A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba*. *Pharmacol. Biochem. Behav.* (2002) 73:893-900.

18. Compilation Board of China Academy of Sciences and China flora. In: China Flora [M]. Science Press, Beijing (1977) 304-48.

19. Harley RM, Atkins S, Budantsev AL, Cantino PD and Conn BJ. In: The families and Genera of Vascular Plants. 7th edn. Springer-Verlag publication, Berlin, (2004) 167–275

20. Barua CB, Bora M, Saikia BN, Hazarika M, Misri J and Barua IC. *Int J Pharm. Bio. Sci.* (2015) 6:539-47.

21. Madrigal JL, Olivenza R, Moro MA, Lizasoain I, Lorenzo P and Rodrigo J. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. *Neuropsychopharmacol.* (2001) 24:420-9.

22. Sahin E and Gümüşlü S. Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. *Behav. Brain Res.* (2004) 155:241-8.

23. Porsolt RD, Deniel M and Jalfre M. Forced swimming in rats: hypothermia, immobility and the effect of imipramine. *Eur. J. Pharmacol.* (1979) 57:431-6.

24. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the folin phenol regent. *J. Biol. Chem.* (1951) 193:265-75.

25. Okawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* (1979) 95:351-8.

26. Marklund S and Marklund G. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. *Eur. J. Biochem.* (1974) 47:469-74.

27. Aebi H. Catalase in vitro. *Methods Enzymol.* (1984) 105:121-6.

28. Flohé L and Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol.* (1984) 105:114-21.

29. Sheikh N, Ahmad A, Siripurapu KB, Kuchibhotla VK, Singh S, Palit G (2007) Effect of *Bacopa monniera* on stress induced changes in plasma corticosterone and brain monoamines in rats. *J Ethnopharmacol.* (2007) 111:671-6.

30. Woodward CJ and Emery PW. Determination of plasma corticosterone using high performance liquid chromatography. *J. Chromatogr.* (1987) 419:280-4.

31. Ipek Y and Fazilet A. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *Eur. J. Pharmacol.* (2005) 514:165-74.

32. Omoregie ES and Osagie AU. Effect of *Jatropha tanjorensis* leaves supplement on the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats. *J. Food Biochem.* (2011) 35:409-24.

33. Sheikh N, Ahmad A and Siripurapu KB. Effect of *Bacopa monniera* on stress induced changes in plasma corticosterone and brain monoamines in rats. *J. Ethnopharmacol.* (2007) 111:671-6.

34. Garrido P. Aging and stress: past hypotheses, present approaches and perspectives. *Aging Dis.* (2011) 2:80-9.

35. Kulkarni MP and Juvekar AR. Attenuation of acute and chronic restraint stress induced perturbations in experimental animals by *Nelumbo nucifera* Gaertn. *Indian J. Pharm. Sci.* (2008) 70:327-32.

36. Tache Y and Selye H Shift in adenohypophyseal activity during chronic intermittent immobilization of rats. *Neuroendocrinol.* (1976) 22:325-36