THE EFFECT OF *ECHINACEA ANGUSTIFOLIA* ON VARIOUS BIOCHEMICAL PARAMETERS IN STRESS INDUCED RATS

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Summary

The present study was carried out to evaluate the effect of ethanolic root extract of *Echinacea angustifolia* on swimming endurance and anoxia tolerance test in mice, cold induced stress and Immobilization in albino rats. The effect was assessed by swimming survival time and anoxia tolerance time, estimation of various biochemical parameters in cold and immobilization stress like glucose, cholesterol, triglycerides, plasma cortisol and blood urea nitrogen (BUN), and by determining the weight of organ such as, liver, spleen and adrenal gland. Blood cell count (WBC) and also the differential count at a dose of 200mg/kg and 400 mg/kg body weight per oral was carried out. It was found that extract significantly ($p<0.001$) increases swimming time and anoxia tolerance time. It also showed significant ($p<0.001$) decrease in blood glucose, cholesterol, triglyceride, plasma cortisol and BUN levels and also showed a significant ($p<0.05$) decrease in weight of organs. A significant ($p<0.01$) decrease in WBC count, polymorphs and monocytes and decrease in lymphocytes ($p<0.05$) and eosinophils was observed, compared to control group. Thus the obtained results revealed that the *Echinacea angustifolia* has got a significant anti stress activity.

Key words: Stress, *Echinacea angustifolia*, WBC, BUN

Introduction

Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis and has been defined as a nonspecific response of the body to any demand imposed on it. Stress is a response to physical, chemical, biological and emotional changes, consisting of a pattern of metabolic and behavioral reactions that helps in strengthening the organism. Herbal medicines are rich in non-specific anti stress agents which are of increasing clinical significance, among them adaptogens are the plant derived biological active substances which increases the power of resistance against physical, chemical or biological noxious agents[1-4].

Echinacea angustifolia known as Black Sampson of Compositae family known to have a great medicinal importance possessing many medicinal properties like, immunomodulator[5], anti-inflammatory[6], anti-microbial[7,8], anti-cancer[8], anti-oxidant[9] and wound healing activity[10]. This study is designed to evaluate anti stress activity of *Echinacea angustifolia*.

Materials and Method

The Ethanolic root extract of *Echinacea angustifolia*(EREA) (ECAG/JA 0071) and Ethanolic extract of *Panax ginseng* (EPG) (PAN-C00531) a gift sample obtained from Madhur Pharma, Bangalore.
The dried ethanolic extracts were suspended in distilled water using 1% Tween 80, used for pharmacological screening.

Experimental animals

Adult Swiss albino mice (20- 25g) and Wistar rats (150 -200g) of either sex were used for the study. The mice and rats were fed with standard pellet (Parnava Agro industries Ltd. Sangali, India) and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. The study protocol was approved by IAEC. (No.CPCSEA/IAEC/PC-01/346)**.**

Acute toxicity study [11]

The study was performed according to the acute toxic classic method (as per CPCSEA/OECD guidelines). Swiss albino mice were used for acute toxicity study. The animals were kept Fasting for overnight providing only water, after which the test drug extract dissolved in water was administered orally at the dose of 1000 mg/kg and observed for 14 days. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24h (with special attention during the first 4h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection urinary incontinence, defecation) and central nervous system (ptosis, drowsiness, gait, tremors, convulsion). The toxicity study carried out as per the guidelines of AOT- 421 using albino mice. The extracts were found to be safe till 1000mg/kg per oral. Hence the dose of 200 and 400mg/kg of EREA was selected for pharmacological screening.

Anoxia stress tolerance test [12]

Albino mice of either sex weighing between (20±2g) were divided into 4 groups of six each. Group I as control, Group II subjected to anoxia test and the oral administration of EREA at the dose 200 mg/kg p.o (per oral), Group III subjected to anoxia test and the oral administration of EREA at the dose 400 mg/kg p.o and Group IV subjected to anoxia test and the oral administration of EPG at the dose 100 mg/kg p.o respectively for 21 days. Anoxia test was carried out on 7^{th}, 14^{th} and 21^{st} day. One hour after the drug administration mice were placed in Hermetic vessel of 2000ml air capacity was used for this test. Each animal was kept in the hermetic vessel and the time to show the first sign of convulsion seen in animal were immediately removed from the vessel and the time noted. After one week of drug treatment the animals were once again exposed to the anoxia stress. Similarly the animals were also observed at the end of 2^{nd} and 3^{rd} week with the same treatment and the time duration for anoxia stress tolerance was noted. The data obtained were subjected to statistical analysis.

Swimming endurance test [13]

Swiss albino mice (20 ± 2 g) of either sex were randomly divided into 4 groups of 6 animals each consisting Group I as control, Group II swimming test and subjected to the oral administration of EREA at the dose 200 mg/kg, Group III swimming test and subjected to the oral administration of EREA at the dose 400 mg/kg and Group IV subjected to anoxia test and the oral administration of EPG at the dose 100 mg/kg p.o respectively for 21 days. Swimming test was carried out on 7th, 14th and 21st day. One hour after the drug administration mice were allowed to swim in rectangular container (24×24×18 cm) filled with water maintained at 25 ± 2°C till they got exhausted and the moment they drowned head was considered as the endpoint. The time was noted and the data obtained were subjected to statistical analysis.

Cold stress

Albino rats (210-230gm) of either sex were divided in to 5 groups of 6 animals each. Group-I served as control, Group-II served as cold stress control, Group-III cold stress induced and treated with EREA 200mg/kg p.o, Group-IV cold stress induced and treated with EREA 400mg/kg p.o and Group-V cold stress induced and treated with EPG 100mg/kg p.o. Cold Stress was induced in 2nd, 3rd, 4th and 5th groups in albino rats, by exposing animals to 4 ±1°C daily for 2 hrs for10 days[14]. On 11th day all the animals were sacrificed and blood was collected for estimation of biochemical parameters like, glucose, cholesterol, triglycerides, plasma cortisol and BUN[15], blood cell count[16] and weight of organs such as liver, spleen and adrenal gland [17].

Immobilization stress

Albino rats (210-230gm) of either sex were divided into 5 groups of 6 animals each. Group-I served as control, Group-II served as immobilization stress control, Group-III immobilization stress and treated with EREA 200 mg/kg, Group-IV immobilization stress and treated with EREA 400mg/kg and Group-V immobilization stress treated with EPG 100mg/kg. Immobilization stress was induced in 2nd, 3rd, 4th and 5th group animals by immobilizing the animals with head down in supine position, by fixing the animals to a board inclined position at an angle of 60° daily for 2 hrs for the duration of 10 days[18,19]. On 10th day, the animals were sacrificed and blood was collected for estimation of biochemical parameter like glucose, cholesterol, triglyceride, Plasma cortisol and BUN levels[15], blood cell count[16] and weight of organs like liver, spleen and adrenal gland[17].

Statistical analysis

All the values are expressed as mean ±SEM and data was analyzed by one-way ANOVA, using Graph pad INSTAT. The post-hock analysis was carried out by Dunnet's multiple comparison test to estimate the significance of difference between individual groups.

Results

The Anoxia tolerance test was determined by taking the appearance of convulsion as end point. EREA at the dose of 200 and 400mg/kg body weight has shown significant ($p<0.001$) increase tolerance stress time in 14th and 21st day compared to control (Table 1).

In swimming endurance test EREA at the dose of 200 and 400mg/kg body weight has shown significant ($p<0.001$) increase in swimming time compared to control (Table 2).

In cold stress induced, EREA has significantly ($p<0.001$) reduced the elevated levels of biochemical parameters like glucose, cholesterol, BUN, triglyceride and plasma cortisol levels compared to stress control group (Table 3). EREA has also reduced the blood cell count WBC's significantly ($p<0.01$) and lymphocytes and eosinophils ($p<0.05$) compared to stress control group (Table 4). Determination of weight of organs showed that EREA has significantly ($p<0.01$) reduced the weight of liver, spleen and adrenal gland (Table 5).
In immobilization stress induced, EREA has significantly ($p<0.001$) reduced glucose, cholesterol, BUN, triglyceride and plasma cortisol when compared with stress control group (Table 6). It has also reduced the blood cell count WBC's significantly ($p<0.01$) compared to stress control group (Table 7). Determination of weight of organs showed that the extract has significantly ($p<0.01$) reduced the weight of liver, spleen and adrenal glands (Table 8).

Table 1: Effect of EREA on anoxia tolerance test

Group	Mean duration of tolerance time (in min) Mean± SEM		
	1st week	2nd week	3rd week
Control	3.8 ± 0.15	6.5 ± 0.65	11.8 ±0.15
EREA 200mg	3.2±0.71*	5.9 ±1.35***	8.5 ± 0.22**
EREA 400mg	3.7±0.18*	6.2 ±1.07***	10.2 ± 0.11**
EREA 100mg	3.3 ±0.11*	6.7 ± 0.25**	11.3 ± 0.83**

The values are expressed as mean ± S.E.M, n=6.
Significance at *(P<0.05), ** (P<0.01), *** (P<0.001), when compared to control.

Table 2: Effect of EREA on swimming endurance test

Group	Mean duration of Swimming survival time (in min) Mean± SEM		
	1st week	**2nd week**	**3rd week**
Control	12.6 ±1.74	18.2 ±0.73	24.8 ±1.55
EREA 200mg	11.8±0.18**	15.8 ±1.07**	19.4± 1.09***
EREA 400mg	12.5± 0.73*	19.3 ±1.17***	22.4 ± 0.14**
EPG 100 mg	12.2 ± 0.18*	18.8 ± 0.98**	22.5 ± 0.83**

The values are expressed as mean ± S.E.M, n=6.
Significance at *(P<0.05), ** (P<0.01), *** (P<0.001), when compared to control

Table 3: Effect of EREA on biochemical parameters in cold stress

Parameter	Control	Cold stress	EREA 200mg	EREA 400mg	EPG 100mg
Glucose mg/dL	82.54 ± 0.762	115.2 ±1.92	96.55 ±1.15***	84.25 ±2.22***	83.32 ± 3.21
Cholesterol mg/dL	42.41 ± 1.82	58.5 ±1.52	43.15 ±2.03***	41.24 ± 1.72***	44.51 ± 2.15
Triglyceride mg/dL	72.44 ± 0.52	106.6 ±2.74	84.24±2.05***	70.44 ± 0.65***	74.51 ± 1.65
BUN mg/mL	32.14 ± 0.52	54.45 ±2.51	40.17±1.32***	31.88 ± 0.92***	34.14 ± 1.24
Plasma cortisol (µg/100 ml)	14.08 ± 0.25	22.45±0.66*	19.64±0.54*	16.61±0.09**	15.92±0.34

Table 4: Effect of EREA on weight of organ in cold stress

Parameter	Control	Cold stress	EREA 200mg	EREA 400mg	EPG 100mg
Spleen mg/100g	3.15 ±0.11	2.31±0.12	3.08±0.21**	3.31±0.25**	3.32 ± 0.26**
Liver g/100g	5.142±62.5	6.718±38.2	5.921±13.8***	5.22±11.8***	5.101 ± 10.8**
Adrenal gland g/100g	0.255±0.01	0.488±0.45	0.358±0.01**	0.281±0.01**	0.272 ± 0.01**

Table 5: Effect of EREA on blood cell count in cold stress

Parameter	Normal	cold stress	EREA 200mg	EREA 400mg	EPG 100mg
WBC	4844 ± 40.22	6670 ± 25.1	6014 ±33.1***	5123±42.21***	5204±46.01
Lymphocytes	47 ± 1.52	68 ± 1.22	60 ± 0.12**	55 ± 1.03**	54 ± 1.02
Monocytes	1.00 ± 0.0	3.15 ± 0.19	1.45 ± 0.50**	1.5 ± 0.46**	1.5 ± 0.71
Neutrophills	23 ± 0.41	36 ± 0.85	27 ± 0.50*	29 ± 0.22*	28 ± 0.01
Eosinophils	1.24 ± 0.2	4.55 ± 0.22	2.25 ± 0.15*	1.0 ± 0.10*	1.0 ± 0.25

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Table 6: Effect of EREA on Biochemical parameter in immobilization stress

Parameter	Control	Cold stress	EREA 200mg	EREA 400mg	EPG 100mg
Glucose mg/dL	81.44 ± 0.83	112.5 ±2.51	95.55 ±5.11***	84.55 ±4.22***	83.25 ± 3.21
Cholesterol mg/dL	41.23 ± 1.88	59.8 ±1.76	44.28 ±2.53***	40.21 ± 1.03***	40.54 ± 2.65
Triglyceride mg/dL	71.22 ± 0.712	105.4 ±2.14	83.84±1.92***	75.38 ± 0.65***	74.88 ± 1.65
BUN mg/mL	30.54 ± 0.552	54.44 ±2.01	39.08±1.12***	32.88 ± 0.94***	33.58 ± 1.83
Plasma cortisol (µg/100 ml)	12.04 ± 0.24	22.21±0.56*	18.65±1.01*	15.23±0.14**	13.21±0.32

Table 7: Effect of EREA on weight of organ in immobilization stress

Parameter	Control	Cold stress	EREA 200mg	EREA 400mg	EPG 100mg
Spleen mg/100g	3.59 ±0.12	2.59±0.15	3.06±0.22**	3.31±0.22**	3.33 ± 0.26**
Liver g/100g	5.124±14.5	6.715±32.2	5.941±13.5***	5.22±11.8***	5.181 ± 10.8**
Adrenal gland g/100g	0.254±0.01	0.485±0.42	0.353±0.01*	0.280±0.01**	0.275 ± 0.01**

Table 8: Effect of EREA on blood cell count in immobilization stress

Parameter	Normal	cold stress	EREA 200mg	EREA 400mg	EPG 100mg
WBC	4844 ± 41.01	6940 ± 45.9	6014 ± 40.2***	5521±42.21***	5216±45.21
Lymphocytes	47 ± 1.74	69 ± 1.20	64 ± 0.52**	56 ± 1.03**	55 ± 1.04
Monocytes	1.00 ± 0.0	3.35 ± 0.15	1.55 ± 0.50**	1.5 ± 0.46**	1.5 ± 0.04
Neutrophills	24 ± 0.42	35 ± 0.88	28 ± 0.50*	30 ± 0.22*	29 ± 0.91
Eosinophils	1.24± 0.5	4.51 ± 0.22	2.24 ± 0.25*	1.82 ± 0.05*	1.41 ± 0.25

The values are expressed as mean ± S.E.M, n=6.
Significance at *(P<0.05), ** (P<0.01), *** (P<0.001), when compared to stress control.

Discussion

Stress produce characteristic changes in several hormones and parameters associated with the central nervous system and the hypothalamic-pituitary-adrenal axis (HPA). HPA changes include an increase in cortisol, a reduced sensitivity of the HPA to feedback down-regulation, and a disruption in the circadian rhythm of cortisol secretion. Central nervous system changes include the stress-induced depletion of catecholamine neuro transmitters such as nor epinephrine and dopamine. An acute increase in beta-endorphin levels is also observed under stressful conditions [20].

Rodents when forced to swim in a restricted space become immobile after an initial period of vigorous activity indicating the stress. Pretreatment with adaptogen increase swimming endurance in mice[21]. Increase in total swimming time of EREA treated mice showed significant improvement in the swimming time. Cold stress typically increases total leukocyte count, eosinophils and basophils. Plant adaptogen are smooth prostressors which reduce the reactivity of host defense system. The mode of action of adaptogens is basically associated with stress system. Adaptogens increase the capacity of stress to respond to the external signals of activating and deactivating mediators of stress response subsequently[21, 22]. The stress induced increase in total WBC count, which is decreased by EREA indicating its antistress and adaptogenic activity are similar to the changes produced by reference drug EPG.

Release of ACTH in stress stimulates adrenals to increase production of hormones epinephrine, norepinephrine, and corticosteroids. These hormones have profound effect on metabolic functions. Adrenaline raises blood sugar, while corticosteroid stimulates glycogenolysis and gluconeogenesis. Thus, increased cortisol influences mobilization of stored fat and carbohydrate reserve, which in turn increases blood glucose, protein, cholesterol, BUN, plasma cortisol and triglyceride levels[23,24].

All the body functions, including cellular respiration depends on the oxygen supply. Any lack of vital element will play havoc on all body mechanisms and increase in adaptation during stress by any drug could be considered as its major anti stress effect. During stress adaptogens are capable of increasing succinate dehydrogenase[SDH] in the brain, decrease in brain neurotransmitters like norepinephrine(NE), dopamine(DA), serotonin(5-HT) and acetylcholine(ACh). Hence the observed drug effective in this model may be effective by the modulation of above mentioned neurotransmitters[25]. The extract of EREA showed significant increase in anoxia tolerance time which is an indication of either resistance to it or reduction in cerebral oxygen consumption. Both these effect are useful to protect neuronal cell against oxidative stress, the enzyme is responsible for utilization and conservation of energy in the cellular system of the organism; which helps adaptive processes during stress.

EREA as well as the standard drug EPG significantly reduced the elevated serum cholesterol, triglyceride, plasma cortisol and BUN levels which may be due to inhibition of stimulation of sympathetic nervous system.

Organ body weight indices of adrenal and spleen gland were used as vital markers for studying stress response. The increase in adrenals in stressed animals is due to the stress induced adrenomedullary response leading to increased production of corticotropic hormone that leads to increase in weight of adrenals[26]. EREA and EPG has significantly reduced the liver and adrenal gland weight, this might be due to the reversal of stress induced adrenomedullary response and hence decrease production of corticotropic hormone. Pretreatment with EREA and EPG significantly increased the spleen weight. This might be due to the inhibition of recruitment of lymphocytes to blood from spleen.

Stress causes alteration in hematological parameters like increase in WBC and DLC counts, neutrophils[16,26]. EREA as well as the standard EPG significantly reduced the WBC, lymphocytes, eosinophils and monocyte counts in cold and immobilization stress.

A variety of biological activities including Anti-stress activity were reported with flavonoids, tannins and glycosides[26]. *Echinecea angustifolia* contains biologically active chemicals that include glycosides, alkaloids, phenols like echinacoside, Cichoric, caftaric acid, alkylamides, polysaccarides like inulin, flavanoids, tannins and volatile oil[27,28]. The anti stress activity may be due to the presence of these constituents where as standard drug *Panax ginseng* an established adaptogenic drug too contains glycosides, steroids and flavoniods[29].

Conclusion

The results obtained from this study suggest that the Ethanolic extract of *Echinacea angustifolia* has potential Anti-stress activity which is comparable to standard Ethanolic extract of *Panax Ginseng*.

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