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Adaptogenic and anti-amnesic properties of *Evolvulus alsinoides* in rodents

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Abstract

Evolvulus alsinoides (EA) is well known for its memory enhancement, antiepileptic and immunomodulatory properties in the traditional Indian system of medicine, Ayurveda. In view of the increasing attention towards plants offering non-specific resistance (adaptogens) towards stress, we have evaluated crude ethanolic extract of EA for its adaptogenic and memory enhancing properties in rodents. Adaptogenic activity was assessed in rats subjected to acute and chronic unpredictable stress. Male Sprague–Dawley rats, weighing 180–200 g were immobilized for 150 min once only in acute stress (AS) model, whereas in chronic unpredictable stress (CUS) model rats were subjected to different types of stressors daily for 7 days. Stress exposure has induced gastric ulceration with increase in adrenal gland weight, plasma creatine kinase (CK), and corticosterone level in AS and CUS. However plasma glucose was increased only in AS. Rats were treated with graded doses of crude ethanolic extract of EA (100, 200 and 400 mg/kg p.o.) for 3 days and subjected to AS on 3 day after 45 min of last dose. In CUS, EA at a dose of 200 mg/kg p.o. found effective in acute studies was administered 45 min prior to stress regimen for 7 days. EA reduced the stress induced perturbations similar to *Panax quinquefolium* (PQ) (100 mg/kg p.o.), a well known adaptogen. EA (100 mg/kg) administered orally for 3 days in adult male Swiss mice, was effective in decreasing scopolamine induced deficit in passive avoidance test. The improvement in the peripheral stress markers and scopolamine induced dementia by EA in the present study indicates the adaptogenic and anti-amnesic properties of EA.

Keywords: *Evolvulus alsinoides*; Adaptogen; Corticosterone; Anti-amnesic

1. Introduction

Temporal prolongation of adaptation response to discrete stressful events is characterized by hyperactivity of hypothalamic–pituitary–adrenal (HPA) axis. Sustained hyperactivity of the stress system (HPA axis) results in various pathophysiological states that cut across the traditional concept of disease and include a range of disorders like hypertension, coronary heart disease (Roy et al., 2001), gastric ulcers (Yadin and Thomas, 1996), immunosuppression (Purett, 2001), metabolic disorders like diabetes (Fitzpatrick et al., 1992), reproductive dysfunction (Dabson and Smith, 2000), mental depression, memory loss and host of other diseases (Gareri et al., 2000). Due to the non-specific nature of the stress pathogenesis, a separate class of therapeutic agents was evolved known as "adaptogens". The term adaptogen was described by Lazrev (1947) as "the substance which can develop a state of raised resistance", enabling an organism to cope with stressful situations. Therapeutic approach for stress from ancient times has involved utilization of substances from natural origin, rather than synthesis of new chemical compounds. Pharmacolog-

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ical investigations have shown that the basic effect of *Panax ginseng*, *Elutherococcus senticosus* and *Rhodiola rosea*, is their ability to increase non-specific resistance of the organism to various untoward influences (Brekhman and Dardymov, 1969). Initial studies on plants originating from folk medicine along with an exponential increase in knowledge regarding the interactions among components of the stress system have encouraged various investigators to evaluate the potential of plant adpatogens for usage in modern day medicine. Further enrichment of the study on plant derived adaptogens was enabled by the substantial work carried out on plants such as *Ocimum sanctum* (Bhargava and Singh, 1981), *Emblica officinalis* (Rege et al., 1986), *Bacopa monniera* (Rai et al., 2003a), *Ginkgo biloba* (Rai et al., 2003b) and *Withania somnifera* (Muruganandam and Bhattacharya, 2003). *Evolvulus alsinoides* (EA) is an important plant that has been well documented in Ayurveda for its therapeutic values. EA (Linn) (Family: Convolvulaceae) commonly known as *Shankhpuspi* is found throughout India ascending to 6000 ft in the Himalayas. It is well known for its therapeutic effect on brain disorders like insanity, epilepsy, memory enhancement and nervous debility in Indian Ayurvedic system of medicine (Chatterjee, 1990). Recent pharmacological studies on leaves and whole plant of EA have indicated anti-ulcer (Asolkar et al., 1992), immunomodulatory properties (Lilly et al., 2003) and in vitro experiments (Mukherjee et al., 2003) have revealed the anti-oxidant properties of EA. The multifunctional nature of EA made it rationale to select for our present study to evaluate its adaptogenic potential. Considering the diversity of stress disorders, our study has been aimed at understanding the effects of EA on stress induced changes on corticosterone, gastric ulcer, glucose and CK. The adaptogenic property was compared with *Panax quinquifolium* (PQ) commonly called *American ginseng* used in contemporary medicine. Memory is one of the important indices of efficient brain function and is controlled by the brain limbic system, which is intimately involved in the stress response. (Fuchs and Flugge, 2003). There fore EA was also evaluated against scopolamine induced memory deficit.

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (180–200 g) and male Swiss mice (25–30 g) were obtained from National Animal Laboratory Centre, CDRI, Lucknow. Animals were kept in raised mesh bottom cages to prevent coprophagy in environmentally controlled rooms (25±2 °C, 12 h light and dark cycle), animals had free access to standard pellet chow and drinking water except during experiments. Experiments were conducted between 09:00 and 14:00 h.

Experimental protocols were approved by our institutional ethical committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), which complies with international norms of INSA.(Indian National Science Academy).

2.2. Plant material

Whole plant of about 18.0 kg was collected from 24 Parganas, West Bengal, India in the month of September. It was identified in the Division of Botany, CDRI and preserved with voucher specimen number 2659 in the herbarium of the institute.

2.3. Preparation and administration of drugs

Whole plant of EA was dried in shade and powdered. 300 g of powdered whole plant was placed in glass percolator with 95% ethanol (1.5 L) and was allowed to stand at room temperature for about 16 h (overnight). The percolate was collected. This process of extraction was repeated four times. The combined extract was filtered and concentrated under vacuum at 40 °C, and the weight of extract obtained was 18 g.

Aqueous suspension of crude ethanolic extract of EAin 1% sodium carboxy methyl cellulose was prepared and administered orally at a dose of 200 mg/kg once daily for 3 days in case of AS and for 7 days in case of CUS 45 min prior to stress session. Control animals received an equivalent volume of vehicle for the same period. After stress regimen, animals were sacrificed by decapitation on 7th day immediately after last stress session.

The crude powder of ginseng root *P. quinquifolium* was purchased from Sigma, USA (Cat. No. G.7253) and freshly prepared aqueous suspension was used.

For cognition studies aqueous suspension of crude ethanolic extract of EA at a dose of 100 mg/kg p.o. was administered daily for 3 days in mice. Scopolamine at a dose of 3 mg/kg i.p. was used to induce retrograde dementia.

2.4. Experimental methods

2.4.1. Stress protocol

The rats were divided into non-stress (NS), AS, CUS groups and drug treated groups for both AS and CUS groups. Each group consists of 6 rats. In AS model, 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, rats were stressed. A parallel group of vehicle treated rats with out exposure to any kind of stress and maintained under normal conditions served as control non-stress group. In CUS the drugs were feed daily 45 min prior to stress regime for 7 days except the rats were fasted over night on the sixth day after completion of the experimental regimens of drug feeding and stress exposure.

AS was produced by immobilizing animals for 150 min once only and sacrificed immediately. CUS was produced with variable stressors with modifications as described by (Katz et al., 1981). The stressors include immobilization, forced swimming, soiled cage bedding, foot shock, day night reversal and fasting. Animals were subjected to two stressors of variable intensity every day in an unpredictable manner to avoid habituation for 7 consecutive days. (Rai et al., 2003c).

Briefly, immobilization stress was produced by restraining each naive animal inside an acrylic hemi cylindrical plastic tube (4.5 cm diameter, 12 cm long) for 150 min. In swimming stress animals were allowed to swim in glass jar (35.5 cm long, 20.2 cm diameter) containing water at 25 °C for 20 min. In soiled cages, the bedding was wet with water to produce overnight inconvenience. In foot shock stress animals were subjected to foot shock (2 mA) in agressometer (Techno electronics, Lucknow, India) for 20 min on a grid floor with a shock interval of 2 s. Day and night reversal was produced by keeping the animals in the dark during day and in high intensity light during the night.

2.4.2. Passive avoidance test

The mice were subjected to single trial passive avoidance test as described by Das et al. (2002). Briefly passive avoidance test was performed by computerized shuttle box (Columbus Instruments, USA) provided with a software program PACS 30. An automated guillotine door isolated the compartment lighted at intensity of 8 (scale of 0-off and 10-brightest provided in the PACS 30 software) from the dark compartment. After an acclimatization period of 30 s, the guillotine door automatically opens and the animal is subjected to a trial of 270 s. An entry into the dark compartment automatically shuts of the door and the subject is punished with a single low intensity foot shock (0.5 mA; 5 s). Infra red sensors monitor the transfer from one compartment to another, which is recorded as transfer latency (TL) in seconds. TL was recorded for 1st trial (Acquisition) and next day 2nd trial (retention). The criterion for successful learning and memory activity was taken as an increase in TL on second trial (retention) as compared to first trial (acquisition). Administration of scopolamine (3 mg/kg i.p) 5 min prior to first trial in all the extract treated as well as vehicle treated control groups ($n=5$) was done to induce memory deficit (Amnesia). Another control was of non-scopolamine treated group ($n=5$). The animals were exposed to the first trial 1 h after the last administration of EA on day 3.

2.4.3. Gastric ulceration

Immediately after the stress session rats were killed by decapitation, the stomachs were split open along the greater curvature and the number of discrete ulcers was noted by using magnascope under 5× magnification ulcers were scored according to the method of Gupta et al. (1981), and mean ulcer severity score was calculated.

2.4.4. Biochemical estimations

The blood was collected in EDTA coated tubes, through cardiac puncture after the stress regime and centrifuged at 2000 rpm × 20 min at 4 °C and plasma was separated. The plasma was used to estimate corticosterone, glucose, creatine kinase (CK).

2.4.4.1. Estimation of corticosterone. An HPLC/UV system (Waters, USA) was used for quantification of plasma corticosterone by the method of (Wood ward and Emery, 1987) with modifications. Dexamethasone was used an internal standard. The mobile phase consisted of methanol: water (70:30) at a flow rate of 1.2 ml/min and corticosterone was detected at 250 nm using UV detector. The chromatogram was recorded and analyzed with Breeze soft ware (3.20 version).

2.4.4.2. Estimation of glucose and CK. Auto analyzer (Synchron Cx-5, Beckman) was used to estimate glucose and CK with their respective kits (Beckman Coulter International, Nyon, Switzerland).

2.4.5. Statistical analysis

Mean and S.E.M. were calculated. The data was analyzed using one-way analysis of variance (ANOVA) followed by Student–Newman–Keul's multiple comparison test. Data of ulcer was analyzed by non-parametric ANOVA followed by Dunn's multiple comparison tests. $P<0.05$ was considered to be statistically significant.

3. Results

In our pilot studies, effect of graded doses of EA (100, 200 and 400 mg/kg p.o.) were studied in AS model on markers of stress such as adrenal gland weight, glucose and CK of rat. From these initial studies it was observed that EA was significantly effective at a dose of 200 mg/kg p.o. However higher dose (400 mg/kg p.o.) was not found to bring any significant changes in comparison to its immediate lower dose. Similarly, the lower dose (100 mg/kg p.o.) was ineffective to normalize AS induced perturbations. Therefore EA at a dose of 200 mg/kg p.o. was selected for further studies (Table 1).

3.1. Effect of EA and PQ on adrenal gland weight and mean ulcer score

A significant increase ($P<0.001$) in the adrenal gland weight after AS and CUS was observed when compared to NS group. EA and PQ were significantly ($P<0.01$) effective in reducing the stress induced adrenal hypertrophy. AS and CUS exposure produced gastric ulceration signifi-

Table 1
Effect of graded doses of EA on acute stress induced changes in adrenal gland weight, glucose and CK

Groups (dose mg/kg p.o.)	Adrenal gland (mg/kg wt)	Glucose (mg/dl)	CK (mg/dl)
NS	7.8±0.3	87.0±2.3	293.3±5.16
AS+Vehicle	12.23±0.5@	132.8±4.9@	1048±34.7@
AS+EA (100)	11.41±0.6	126.6±11.3	960±56.99
AS+EA (200)	9.06±0.5*	100±4.61*	766.2±38.0*
AS+EA (400)	10.19±0.8	98.55±5.6	758.5±24.5

Mean±S.E.M of changes in adrenal gland weight, plasma glucose and creatine kinase. The stress group was compared with non-stress control group and the drug treated groups were compared with acute stress group. @$P<0.001$ when compared to non-stress control and *$P<0.01$ when compared with acute stress control group.

cantly ($P<0.001$). EA and PQ were significantly ($P<0.05$) effective in reducing AS and CUS induced mean ulcer score.

3.2. Effect of EA and PQ on biochemical changes

AS ($P<0.001$) exposure resulted in increased plasma corticosterone when compared to NS control. Pretreatment with EA ($P<0.05$) and PQ ($P<0.01$) significantly reduced the increase in corticosterone. CUS exposure has also resulted in increased corticosterone ($P<0.001$) which was reverted back with EA ($P<0.05$) and PQ ($P<0.01$) pretreatment.

The plasma CK and glucose levels were increased by AS significantly ($P<0.001$). But the significant hyperglycemic effect observed after AS exposure was not observed in CUS. Prior treatment of EA and PQ were effective ($P<0.001$) in reducing the AS and CUS induced increase in CK levels and AS induced hyperglycemia ($P<0.01$).

3.3. Effect of EA on memory

In passive avoidance task vehicle treated group has shown significant increase in TL (262±28.0 s) on second trial compared to first trial ($P<0.001$). Scopolamine treatment (amnesia) could not produce a significant increase in TL on second trial (106±17.4 s) as compared to first trial. EA treated group exhibited a significant increase in TL (225.635±12 s) on 2nd trial in comparison to first trial as well as compared to amnesia group ($P<0.05$) (Figs. 1–6).

4. Discussion

With the advent of newer techniques for chemical characterization and pharmacological investigations, plant based drugs are receiving much attention. The importance of plants acting on CNS has been reviewed recently by Carlini (2003), emphasizing the role of adaptogens from plant origin. Although synthetic substances are used for organ specific treatment, plants still remain an ideal choice as adaptogens. In the present study, to obtain a complete profile of peripheral and central protective effects of EA against stress, we have focused on the effect of EA on the acute and chronic changes induced by stressful conditions and also its effect on scopolamine induced memory deficit.

A variety of stress situations have been employed in animals to evaluate anti-stress agents. Stress induced effects mainly depend on duration and type of stressors (Tannebaum et al., 2002). Immobilization has been the ideal choice for the induction of stress responses in animals and more specifically, for the investigation of drug effects, on typical stress-related gastrointestinal, neuroendocrine, and immu-

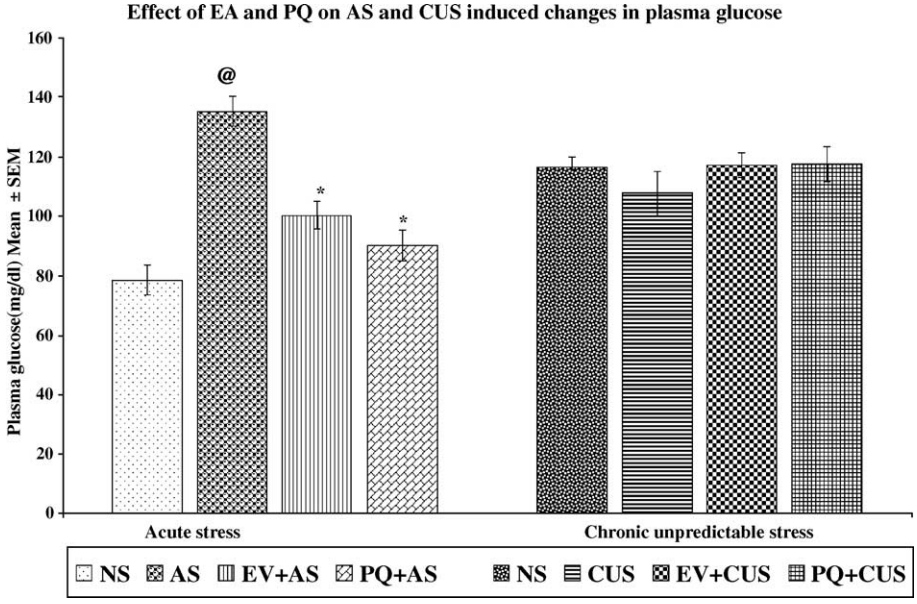

Fig. 1. Bar diagram representing the changes in plasma glucose under AS ($F(3, 20)=21.91$) and CUS for control stress and drug treated groups. The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean±S.E.M. with $n=6$ in each group. @ $P<0.001$ when compared to non-stress control and *$P<0.01$ when compared with acute stress group.

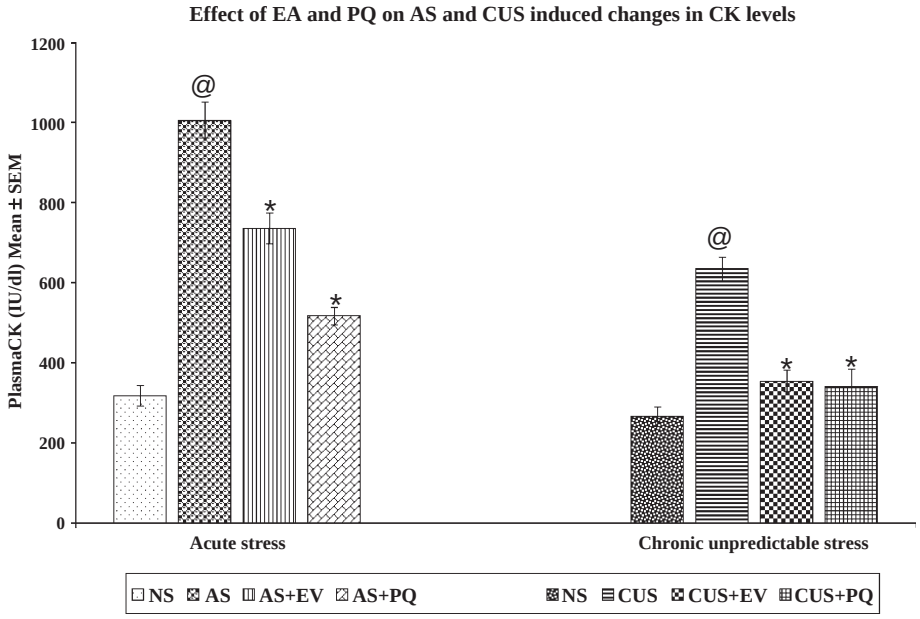

Fig. 2. Bar diagram representing the changes in plasma creatine kinase under AS [$F(3, 20)=122.3$] and CUS [$F(3, 20)=30.34$] for control stress and drug treated groups. The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with $n=6$ in each group. @ $P<0.001$ When compared with non-stress control group.*$P<0.001$ when compared with stress control group.

nological pathology (Glavin et al., 1994). The distinct advantage of using immobilization as a stressor lies in the fact that it produces both physical as well as inescapable psychological stress (Marty et al., 1997) In our study we have selected AS and CUS to evaluate the immediate and chronic anti-stress effects of EA and PQ. The introduction of the factor of unpredictability in CUS model makes it difficult for the individual to adapt and therefore cope with stressors. As such, the use of mild CUS for length of time appears to be more clinically relevant.

Various forms of acute stressful stimuli have been generally accepted to cause hyperglycemic response (Gotoh et al., 2001). This was due to the release of glucocorticoids which is a result of HPA axis stimulation, to compensate the initial demand of energy (Mason, 1968). Hyperglycemic response during stress has been associated with glucose

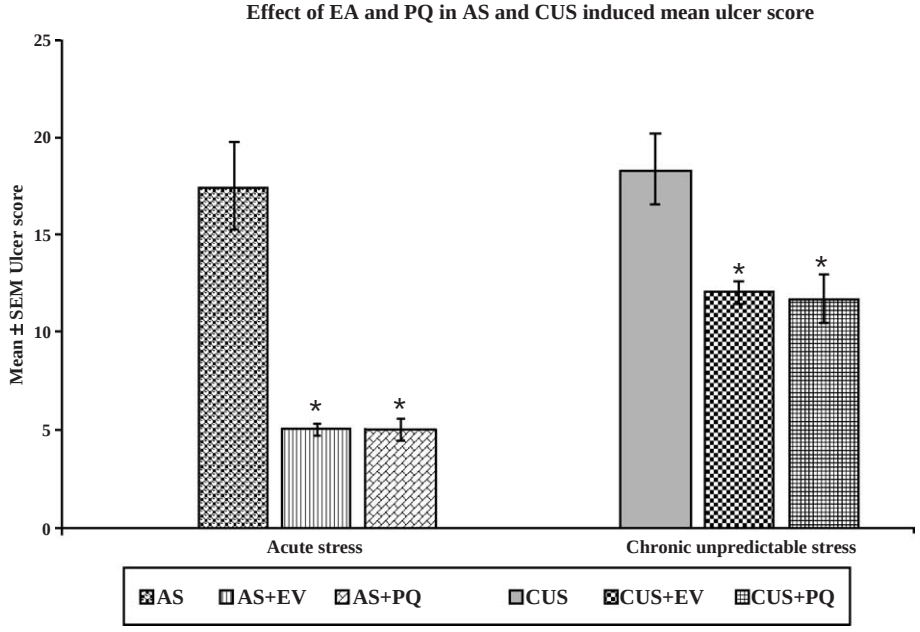

Fig. 3. Bar diagram representing the changes in mean ulcer severity under as and CUS for control, stress and drug treated groups. The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with $n=6$ in each group @$P<0.001$ when compared to non-stress control and *$P<0.01$ when compared to stress control groups.

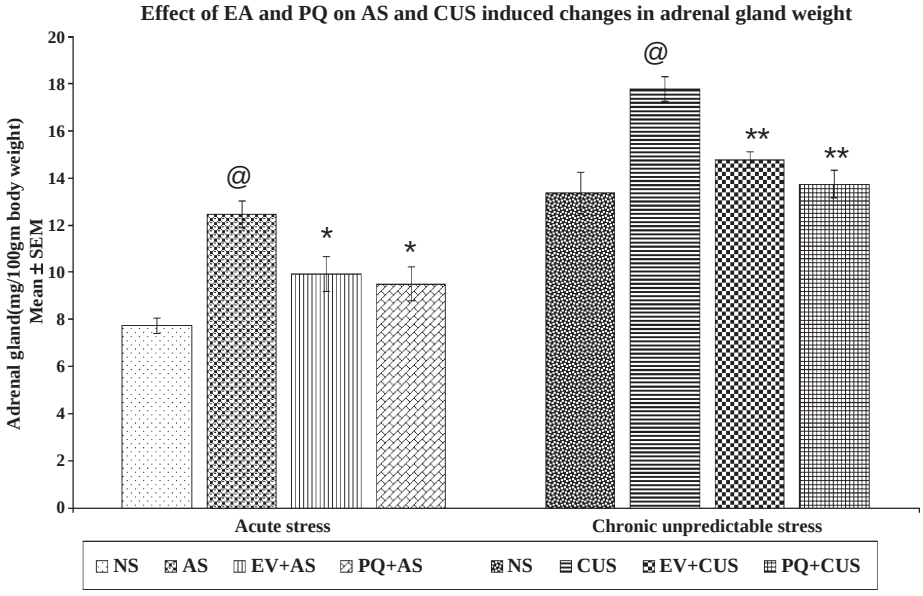

Fig. 4. Bar diagram representing the changes in adrenal gland weight under AS [$F(3, 20)=42.44$] and CUS [$F(3, 20)=33.28$] for control, stress and drug treated groups. The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with $n=6$ in each group. @$P<0.001$ when compared to non-stress control group and *$P<0.05$, **$P<0.001$ when compared with stress control groups.

intolerance (Fukunishi et al., 1998) and subsequently to diabetes. High levels of glucose produce permanent alteration in proteins and increased lipid peroxidation in a variety of experimental models of hyperglycemia (Folmer et al., 2002). AS exposure in our study has elevated the glucose level which is not observed in CUS exposed rats. The early increase in AS cannot be maintained long after the chronic stress procedure (Dal-Zotto et al., 2000) due to the compensation of the energy demand during chronic conditions is from non-carbohydrate origin which are slow and rate limiting (Brindley et al., 1993). Stress hormones not only stimulate and ensure supply of glucose, but also increase CK activity during stress. (Adelbert, 2000). The CK system is important in stabilizing the ATP levels and

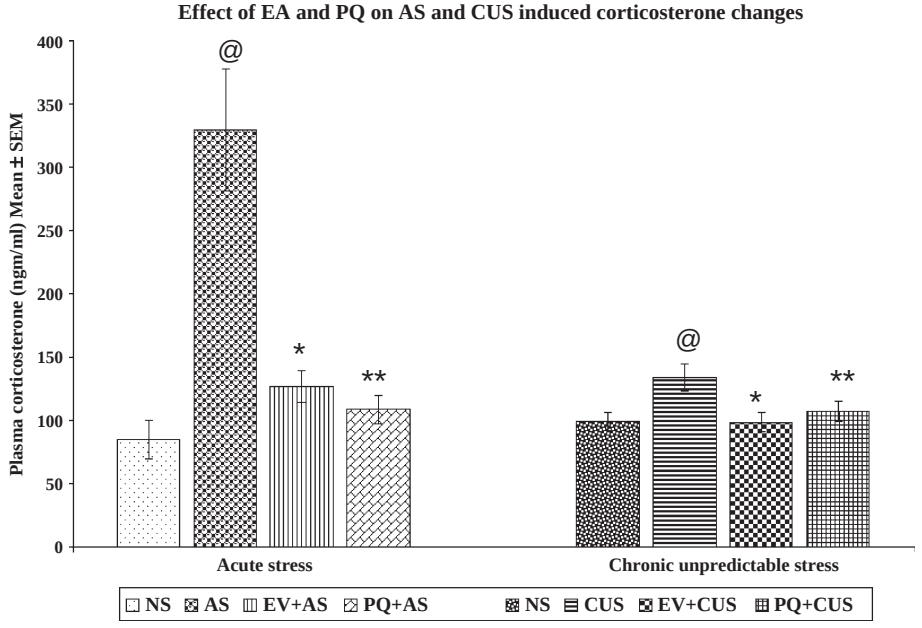

Fig. 5. Bar diagram representing the changes in corticosterone under AS [$F(3, 20)=15.99$] and CUS [$F(3, 20)=10.73$] for control stress and drug treated groups. The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with $n=6$ in each group. @$P<0.001$ when compared to non-stress control group. *$P<0.05$, **$P<0.01$ when compared with stress control.

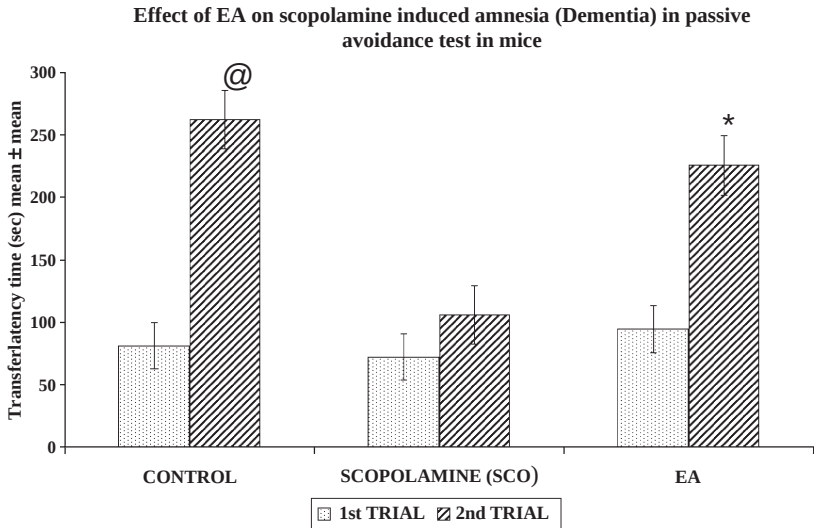

Fig. 6. Bar diagram representing the changes in transfer latency time for control, scopolamine and drug treated groups. The drug treated group was compared with scopolamine treated control group [$F(2, 12)$]=11.34. Results were represented as mean±S.E.M. with $n=5$ in each group. @$P<0.001$ when compared to first trial. *$P<0.05$ when compared to scopolamine treated group.

energy metabolism of the myocardium and other skeletal muscles of rats during stress. CK is known to act as an energy buffer and shuttle between sites of energy production, i.e. mitochondria and ion pumps (Bruton et al., 2003). Perturbations of CK activity during extensive stress may result in ischemia due to the non-availability of ATP (Davydov and Shvets, 2002). A maximum increase in CK activity was observed after AS exposure when compared to CUS. A reduced CK activity in CUS as compared to AS is due to partial habituation which is in accordance with our previous studies (Rai et al., 2003c). EA and PQ treatment have shown immediate decrease in glucose during AS. But they have not shown any change in the glucose level in CUS compared to control rats indicating their anti-hyperglycemic effects only during stressful conditions. The CK level was reduced by EA and PQ in AS and CUS models indicating the decreased energy demand.

Stressful events activate autonomic and endocrine responses (Henke, 1979) responsible for gastric ulceration. In our study AS and CUS induced ulceration in stomach with comparable intensity in both the models. This can be attributed to the stimulation of paraventricular nucleus of hypothalamus, increased intestinal motility, acid secretion and group of other factors (Glavin et al., 1991; Mayer, 2000). Gastric damage induced by CUS and AS has been reduced by EA and PQ as reflected by decreased mean ulcer severity score indicating their protective effects on gastric mucosa during stressful conditions.

Glucocorticoid harmones, mainly corticosterone in rats and cortisol in humans, are the final effectors of the hypothalamic–pituitary–adrenocortical axis and participate in the control of homeostasis and the response of the organism to stressors (Habib et al., 2001). Prominent changes during stress are the adrenocorticoid hypersecretion, increased plasma corticosterone, enlarged pituitary and adrenal size (Dhabar et al., 1997; Makara and Haller, 2001). In our study, AS and CUS have increased the adrenal gland weight and plasma corticosterone. EA and PQ have significantly decreased the adrenal gland weight and plasma corticosterone. But, hyper stimulation of HPA axis results in altered homeostasis leading to various pathological outcomes. Here with the protective effects of EA and PQ in AS and CUS models in our study can be attributed to the decreased activation of HPA axis as shown by the lowered levels of plasma corticosterone which is an immediate and chronic index of hyperactive HPA axis.

Stressful conditions adversely affect cholinergic system and results in learning deficits, but the cholinergic response to the stressful stimuli is variable and depends on the type and duration of the stressor (Pullia et al., 1996). There is substantial clinical evidence that muscarinic receptor blockade by drugs like scopolamine results into disruptions of behavioral inhibition, working (short-term) memory, retrieval from reference (long term memory), attention and decisional processes movement and strategy selection and altered sensory processing (Fibiger, 1990). In the present study, control mice showed significant increase in TL on second trial compared to first trial (acquisition) which shows a successful memory response. Scopolamine treated mice failed to show increase in TL on second trial (retention) indicating deficit in memory (amnesia). However, in mice pretreated with EA, even after scopolamine treatment, a significant increase in TL was observed on second trial (retention). Prevention of scopolamine induced amnesia by EA demonstrated the potential anti-amnesic effect of EA.

Our studies reveal the protective effect of EA on various levels of stress induced perturbations. Further isolation and identification of bioactive constituents of the EA crude extract may provide an effective adaptogenic and memory enhancer.

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