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Research Article

Anti-stress effect of *Centella asiatica* in rats

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Abstract: Stress is considered to cause psychosomatic disorders. A group of plant based drugs, the adaptogens, increase the capacity of body to respond to stressful stimuli. *Panax ginseng*, a known adaptogen was taken as a standard drug. The present study evaluated adaptogenic/antistress potential of high altitude medicinal plant, *Centella asiatica* in a widely accepted immobilization stress model in rats. Male SD rats were exposed to immobilization once for 150 min only in acute stress and 7 consecutive days for chronic stress. This model has been shown to induce significant physiological, biochemical and neurochemical perturbations during acute and chronic stress exposure which could be attenuated by putative adaptogenic agents in rats. After stress, rats were sacrificed immediately, blood was collected and plasma separated for biochemical estimation. The biochemical estimation of plasma glucose, triglycerides, cholesterol, corticosterone and creatine kinase in stressed rats pretreated with *Centella asiatica* at different doses (100, 200 and 400 mg/kg, p.o) showed that it imparted adaptogenic/antistress activity.
Keywords: antistress, *C. asiatica*.

INTRODUCTION

Stress is a major public health problem which has been postulated that stress is involved in the etiopathogenesis of psychosomatic disorders including psychiatric disorders such as depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer; hypertension, ulcerative colitis etc. [1]. Chronic stressful condition associated with altered functions of monoamine neurotransmitters especially noradrenalin, dopamine and 5-hydroxytryptamine [2], which play an important functional role in stressful conditions results in behavioral changes as well as cascade of hormonal release from the hypothalamus–pituitary–adrenal (HPA) axis leading to various disorders like depression, anxiety, obsessive compulsive neuroses, eating and sleeping disorders, hyperglycemia, and decreased immune response. [3,4]. Considerable evidence published in the last decade has focused on alterations of neurochemical, biochemical, and molecular mechanism caused by stress [5-9]. Recent development have reported that the episode of depression is increased 5 to 6 folds following the occurrence of stressful 'life events'. They are considered among the most prevalent psychiatric syndrome, affecting 10% to 30% of general population of industrialized societies [10, 11].

Some plants have been shown to have antistress/adaptogenic activity, the most prominent being *Panax ginseng*, which finds mention in ancient Chinese medicine [12] It has been widely used as an adaptogen, however, it is now known to induce several adverse effects like ''Ginseng abuse syndrome'' on prolonged use [13,14]. Although adaptogens are not officially accepted in modern medicine, but Ayurveda documents several plants, including *Withania somnifera*, *Centella asiatica* etc., which are categorized as Rasayanas. The properties ascribed to rasayanas in Ayurveda are remarkably similar to those of adaptogens. The existing drugs, benzodiazepine anxiolytics, despite having significant antistress activity against acute model of stress, have not proved effective against chronic stress-induced adverse effects on behavior, cognition, peptic ulcer, immunity and hypertension [1]. Furthermore, these drugs have adverse effects on the fetus during pregnancy and on the neonate during lactation [15]. Procedures ranging from yoga and meditation to antistress and anxiolytic drugs like benzodiazepines have been used to counter the aversive effects of stress [16], but appear to have limited use, many patients remain untreated, experience adverse effects of drugs [17,18], or do not get benefited [19]. The present study aims to evaluate adaptogenic/antistress potential of *Centella asiatica*.

Centella asiatica (*Umbelliferae*) is a herbaceous creeping plant widely distributed in Asia including India and South America. In India it is known by a variety of names: Brahmanduki, Brahmi (North India, West India), Gotu kola, Mandukparni, Mandukaparni (South India). According to Ayurvedic Pharmacopoeia, the dried plant is useful in the treatment of a variety diseases including, leprosy, polyurea, pyrexia, dyspnoea, anorexia, anaemia, inflammation, itching and vitiated blood disease (A.P.I. 2004). *CA* has been described by *Charaka* as an "anti-aging herb" and advocated for use in rejuvenation therapy. It is also used to improve memory [20] (Wealth of India, 1992). It has been used in various parts of India for treatment of insanity, asthma and for wound healing [21]. Several other ethnomedical use of the plant has been reviewed by Agarwal (ICMR, Monograph). It has been reported to be used in some central nervous system and gastrointestinal disorders [22]. CA has been found to improve the general mental ability of mentally retarded children [23]. *CA* has been reported to have tranquillizing [24] and anticonvulsant activity [25] and to improve the maze learning in rats [26]. Other reported activities include antitumor [27] antioxidant [28] and antimicrobial [29] activity and an inhibitory effect on the biosynthetic activity of fibroblast cells [30, 31]. *Centella asiatica* has a dose-dependent inhibitory effect on the activity of Ca^{2+}-independent PLA_2 ($iPLA_2$) and cytosolic PLA_2 ($cPLA_2$). The inhibition of these enzymes in the brain suggests that *CA* may be useful to treat conditions associated with increased PLA_2 activity in the brain, such as epilepsy, stroke, multiple sclerosis and other neuropsychiatric disorders. However, no studies have been done regarding the biological efficacy of *Centella asiatica* on various stress responses in experimental animals or in patients.

MATERIAL AND METHOD

Animals

Naïve adult male Sprague-Dawley rats weighing range 180–200 g were used. Animals were housed in a departmental animal house registered under CPCSEA (Reg. No. 1449/GO/q/11/CPCSEA) in a group of 4 in polyethylene cages (38 X 23 X 10 cm) under standard housing conditions (temp., 24 ± 2^0C; humidity, 60-65%) with 12-h light and dark cycle. Food was provided as dry pellets and water was available *ad libitum*. Rats were kept for 7 days in laboratory for habituation before experimentation. Experiments were conducted in accordance with our institutional laboratory animal ethical guidelines.

Collection of plant material and preparation of standardized extract:

Whole plants of *Centella asiatica* were collected from high altitude of district Pithoragarh, Uttarakhand and were identified from the Department of Botany, LSM Govt. Post Graduate College, Pithoragarh. Air dried whole plants of *Centella asiatica* were used for methanolic extraction by percolation. The residue obtained after removing solvent was dried in vacuum and macerated with acetone to give free flowing powder.

Drugs

Panax ginseng (PG) root extract powder was purchased from Sigma (St. Louis, MO, USA), and the standardized extraction from *C. asiatica* was done in the department lab as a test drug.

Drug administration:

The extracts of *P. ginseng*, (100 mg/kg, po) and *C. asiatica* at graded doses (100, 200 & 400 mg/kg, po) (the doses of *C. asiatica* were determined on the basis of the initial pilot studies) was suspended using gum acacia (0.5%) as surfactant to make fine emulsion having uniform particle distribution. The test drug was administered orally daily for 3 days in case of acute stress (AS) and for 7 days in chronic stress (CS). All these drugs were prepared fresh daily before administration.

Experiment procedure:

A number of tests are required to identify and establish the anti-stress properties of a drug [31]. Commonly used test are the swimming endurance test; adrenal functions-weight, ascorbic acid and cortisol content of the adrenal glands; stress induced gastric ulcer; hypoxic convulsions; and cold hypoxia restraint stress (CHR). But immobilization test was used in the present study, because it is widely accepted for studying the stress and can be easily regulated [32, 33].

The rats were divided into control non-stress group, stressed control group and stressed drug treated groups for both AS and CS with 7 rats in each group. AS drug groups were fed with extracts of *P. ginseng* (100 mg/kg, p.o), and test drug *C. asiatica* (100, 200 and 400 mg/kg, p.o) daily for 3 days. In acute stress two parallel groups of rats were fed with vehicle for the same number of treatment days but one group was not stressed while other group was stressed by immobilization. Both these groups were serving as non stress control and stressed control group to obtain base line data for various parameters. Second day after feeding drug or vehicle, animals were fasted overnight with water *ad libitum*. On the third day, 45 min after drug or vehicle feeding, rats were stressed except the non-stress control group. In CS, the drugs were fed 45 min prior to the stress regimen up to seven consecutive days and the rats were kept fasted overnight on the 6th day after drug feeding and stress exposure. A parallel group of non stress control and control stressed group were taken as mentioned above and were sacrificed on seventh day.

The stress was given by restraining the animals inside an adjustable acrylic hemi-cylindrical plastic tube (4.5-cm diameter, 12-cm-long) for a period of 150 min once only in AS and once daily for seven consecutive

days for CS. The rats were sacrificed immediately after stress by decapitation or under ether anesthesia, and the blood was collected from cardiac puncture in EDTA-coated propylene tubes to prepare plasma. The blood was centrifuged (2000 rpm X 20 min at 4^0C) and plasma was separted, stored at -80^0C for biochemical and hormonal assay. The estimation of glucose, triglycerides, cholesterol, and creatine kinase were done with the help of spectrophotometer and corticosterone was estimated by RIA. The adrenal glands were dissected out and weighed after removal of adhering tissues to observe adrenomegaly and histopathology.

STATISTICAL ANALYSIS

Statistical analysis was done using one way ANOVA. *P* values <0.05 was considered significant.

RESULTS

Graded dose (100, 200 and 400 mg/kg, po) of *C. asiatica* were studied in acute and chronic stress and observed biochemical stress parameters (plasma glucose, creatine kinase, plasma triglycerides, plasma cholesterol and serum corticosterone and adrenal gland weight). *P. ginseng* (50, 100 and 200 mg/kg, po) were used as a standard control in stress and non stress control group of rats. *P. ginseng* produced the maximum effect at 100 mg/kg dose and therefore this dose was considered in our study.

Effect of C. asiatica on acute stress induced biochemical alterations

Acute stress induced marked increased in the plasma glucose level (hyperglycemia), plasma creatin kinase, plasma corticosterone and mild increase in plasma triglcerides and plasma cholesterol were observed as compared to non stressed control. Pretreatment with *C. asiatica* at 100, 200 and 400 mg/kg, po reverted the acute stress induced increased plasma glucose, creatin kinase, plasma corticosterone level where as plasma triglcerides and plasma cholesterol level were not significantly reduced except marked reduction in plasma corticosterone at 100 mg/kg, po of *C. asiatica*. Further pretreatment with *Panax ginseng* at the dose of 100 mg/kg, po as a standard significantly reduced plasma glucose plasma creatin kinase and plasma corticosterone while plasma triglcerides and plasma cholesterol were not significantly decreased as compared to non stressed control (Table-1).

Table-1: Effects of *Centella asiatica* on different anti-stress biomarkers in rats in acute stress.

	No stress	Acute Control stressed	100 mg/kg, p.o.(CA)	200 mg/kg, p.o. (CA)	400 mg/kg, p.o. (CA)	100 mg/kg, p.o. (PG)
Plasma glucose (mg/dl)	92.04±6.45	155.11±25.80	132.35±11.29*	135.92±18.33*	117.47±11.37*	103.27±15.87
Plasma Triglycerides (mg/dl)	59.38±4.14	58.07±9.46	54.43±6.42	49.27±9.78	62.92±6.11	48.78±5.22
Plasma cholesterol (mg/dl)	60.76±9.84	66.58±4.75	57.09±6.34	52.52±3.82*	63.98±5.42	56.35±4.49
Plasma Creatine kinase (μ/L)	55.36±9.34	103.33±13.82	73.89±9.56*	81.66±9.58*	90.81±12.26	76.84±6.14
Plasma Corticosterone (nM/L)	77.00±13.72	156.99±29.87	106.9±11.27*	146.045±21.95	149.58±19.74	108.23±15.42
Adrenal gland weight (g/100 g body weight)	0.013±0.0021	0.0204±0.0014	0.0150±0.0014 *	0.0163±0.0020 *	0.0179±0.0027	0.0161±0.0013 *

Values are mean ± SEM; n = 6 per group. *p < 0.05 compared with stress control group.

Effects *C. asiatica* on weight of adrenal gland in acute stress:

A weight of adrenal gland 0.0135 mg/100 g body weight of rat was observed in non stress control group. Stress induced adrenomegaly was observed in stress control group. Pretreatment with *Centella asiatica*, at the dose of 100 and 200 mg/kg (p.o.), produced a marked reduction in the weight of adrenal gland while 400 mg/kg (p.o.) produce a mild reduction as compared to stress control group. *Panax ginseng*, at the dose of 100 mg/kg (p.o) produced a marked decrease in the weight of adrenal gland.

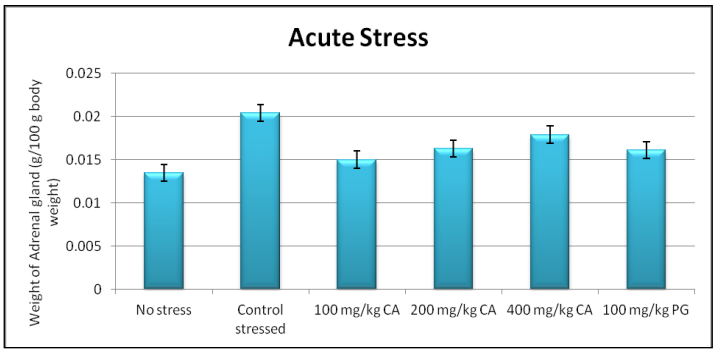

Fig-1: Effects of *Centella asiatica* on weight of adrenal gland in acute stress. Values are mean ± SEM; n = 6 per group. $p < 0.05$ compared with stress control group.

Effect of C. asiatica on chronic stress induced biochemical alterations:

Exposure to chronic stress produced significant increase in plasma glucose, plasma corticosterone and plasma creatine kinase level while marked decrease in plasma triglycerides and moderate decrease in plasma cholesterol were observed as compared to non stress control group. Pretreatment with *C. asiatica* reduced increased plasma glucose level in a dose dependent manner (100, 200 and 400 mg/kg, po), where as 200 and 400 mg/kg, po of *C. asiatica* reduced plasma coricosterone level significantly, plasma creatine kinase was decreased at 200 mg/kg, po and mild decrease in plasma triglycerides was observed at the dose of 200 mg/kg, po of *C. asiatica* as compared to stressed control. *Panax ginseng* at the dose of 100 mg/kg, po effectively decreased all the parameters respectively in comparison to control.

Table-2: Effects of *Centella asiatica* on different anti-stress biomarkers in rats in chronic stress.

	No stress	Chronic Control stressed	100 mg/kg, p.o.(CA)	200 mg/kg, p.o. (CA)	400 mg/kg, p.o. (CA)	100 mg/kg, p.o. (PG)
Plasma glucose (mg/dl)	92.04±6.45	129.52±8.59	121.66±11.29	105.13±18.33*	95.15±10.93*	101.32±12.79
Plasma Triglycerides (mg/dl)	59.38±4.14	39.84±5.85	40.70±2.66	37.87±1.50	29.73±5.99*	31.79±9.72
Plasma cholesterol (mg/dl)	60.76±9.84	51.29±2.03	54.05±4.65	49.51±6.26	38.7±11.17	46.35±5.56
Plasma Creatine kinase (µ/L)	55.36±9.34	84.99±7.93	70.73±5.68	61.64±5.76*	74.811±3.88	69.99±4.16
Plasma Corticosterone (nM/L)	77.00±13.72	142.99±9.60	137.52±10.10	79.49±3.07*	95.52±7.81*	80.49±2.79
Adrenal gland weight (g/100 g body weight)	0.013±0.002	0.026±0.004	0.023±0.002	0.025±0.005	0.020±0.002*	0.018±0.003

Values are mean ± SEM; n = 6 per group. $p < 0.05$ compared with stress control group.

Effects on weight of adrenal gland in chronic stress:

In non stress control group the weight of adrenal gland was observed 0.0135 mg/100 g body weight of rat. Chronic stress induced adrenomegaly and the weight of adrenal gland was measured 0.026 mg/100 g body weight of rat. *Panax ginseng*, at the dose of 100 mg/kg (p.o.) significantly reduced weight of adrenal gland. At the dose of 100 mg/kg, p.o *Centella asiatica* a mild reduction was observed and at the dose of 200 mg/kg, po very slight and almost negligible change in weight of adrenal gland was observed as compared to control group. A marked reduction in the weight of adrenal gland was observed at the dose of 400 mg/kg (p.o).

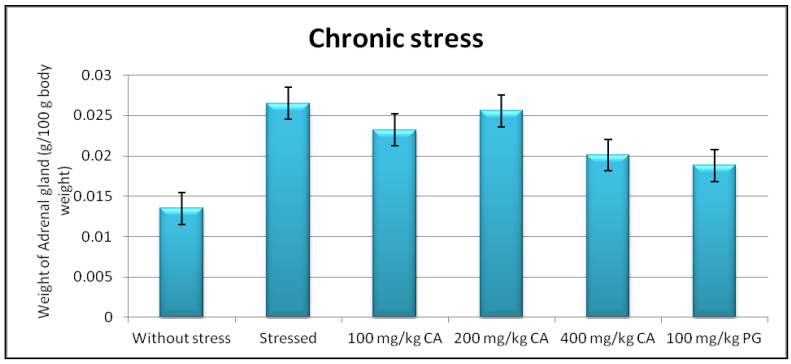

Fig-2: Effects of *Centella asiatica* on weight of adrenal gland in rats. Values are mean ± SEM; n = 6 per group. $p < 0.05$ compared with stress control group.

DISCUSSION

Every individual is likely to face stressful situations in day to day life [34], that may ultimately lead to compromised health. Increased incidence or exacerbation of several neurological and psychological disorders (depression, anxiety, post-traumatic stress disorder distress, despair) emotional strain (frustration dissatisfaction, tension, etc.) maladaptive behaviors (e.g., aggression, substance abuse), often results from chronic psychological stress [35]. Considerable evidence suggests and concentrates on alterations in neurochemical, biochemical and molecular effect caused by stress in these systems [5, 6, 7, 8, 9].

Stress induced changes are initially compensatory, self limiting and adaptive. The changes become rather irreversible and may get pathological in nature when stress events override certain "threshold" limits [36]. It has been postulated that stress is involved in the etiopathogenesis of psychosomatic disorders including psychiatric disorders such as depression and anxiety; immunosuppression; endocrine disorders including diabetes mellitus, male sexual dysfunction; cognitive dysfunctions; peptic ulcer; hypertension, ulcerative colitis etc. [1]. Prolonged stressful conditions are sub-served by a complex system which is associated with the involvement of hypothalamus–pituitary–adrenal (HPA) axis [37, 38, 39]. Potential disturbances from internal or external sources (stressors), which are collectively perceived by the individual as "stress" induce alterations in monoaminergic activity [2] resulting in behavioral changes as well as a cascade of hormonal release from the hypothalamus–pituitary–adrenal (HPA) axis leading to depression, anxiety, obsessive compulsive neuroses, eating and sleeping disorders, hyperglycemia, decreased immune response and prolonged activation of PVN of hypothalamus which causes increased ulcer index. [3, 4, 40].

HPA axis dysregulation caused by stress results in excess production of noradrenalin and corticosterone, that sensitizes peripheral inflammatory response [41], and increases anxiety [42]. Cognitive deficits, social deficits [42], and neuronal death in the hippocampus [41, 42, 43] have also been reported. Repetitive stress exposure also leads to enhanced release of neuropeptides in the brain, such as vasopressin and corticotrophin releasing hormone (CRH) [44]. The raised level of catecholamines, neuropeptides and corticosterone gradually change the electrical properties, morphology and proliferative capacity of brain cells, thus giving rise to the central and behavioral components of stress response. Repetitive exposure to uncontrollable stressors is known to be a risk factor for the precipitation of psychiatric disorders in vulnerable individuals [45]. Stress and stress hormones (corticosterone) also affect other aspects of brain function, such as bio-availability of neurotransmitters [46] and metabolic processes [47], each contributing to the dynamic range of cellular effects of stress, thus affecting normal functioning of psychological and physiological processes.

In response to acute stress, the sympathetic nervous system results in secretion of cortisosterone and epinephrine from adrenal cortex and medulla [56, 57]. These hormone cause hyperglycemia and an increase in the creatine kinase level to provide substrate for energy metabolism in muscle, CNS and organs of demand. These hormones are considered necessary for the body against stress response and also in combating stress. Increased levels of stress hormones and sympathetic innervations have profound effect on metabolism. Hyperglycemic effect of these stress have been reported by many authors [48, 49, 50]. The exogenous administration of corticosterone (dexamethasone) has been reported to induce hyperglycemia [51] and insulin resistance independent of glucose transport [52, 53]. Adrenal hypertrophy and gastric ulceration found in acute and chronic stress indicates prolonged activity and HPA involvement to stress and it is considered as one of the principal mechanism on which an organism mobilizes its defence against stress events [54, 55].

The decreased mucosal blood flow and hyper contractibility during stress is due to the hyperactivation of PVN of hypothalamus causes pathogenesis of gastric ulcers. [40]. It is evidenced that adrenal hypertrophy

occurs in response to ACTH secretion from the pituitary for enhanced corticosterone to combat stress [57].

The higher level of corticosterone in acute stress as compared to chronic stress indicates that chronic stress may alter biochemical and endocrinological parameters in different manner. The enhanced plasma glucose and keratin kinase have been observed more prompt in acute stress than chronic. This prominent effect that was observed in acute stress may be due to the adaptation to stress. It is evidenced that during acute stress increased level of glucose and creatine kinase is required for maintaining ATP availability to muscle and CNS, which decrease during chronic stress exposure [49, 58]. Studies have also reported that hyperglycemic effect of corticosterone is due to enhanced glycogenolysis of glycogen in liver during acute stress [49]. The stored glycogen depletion during chronic stress initiate glyconeogenesis in response to corticosterone [59] for which the levels of cholesterol and triglyceride were decreased during chronic stress exposure.

The pre-treatment of *C. asiatica* decreased the acute stress induced adrenal hypertrophy, hyperglycemia, and creatin kinase level at lower doses, but higher dose did not reduce creatin kinase and adrenal gland weight except plasma glucose. This clearly shows its potent antistress activity. The decrease in the adrenal gland weight indicates its potential role in attenuating the activation of HPA axis. The involvement of HPA axis during stress causes adrenal enlargement and metabolic changes. *Centella asiatica* reduced the acute stress induced plasma glucaose, adrenal gland weight, creatine kinase and plasma corticosterone indicated its action on HPA axis.

Prolonged activation of HPA axis occurs during chronic stress, thus noticeable effect on adrenal gland weight, enhanced plasma glucose, corticosterone was observed. Pretreatment with *Centella asiatica* extract attenuates chronic stress induced enhanced adrenal gland weight; enhance plasma glucose, corticosterone at higher doses. This indicates that *Centella asiatica* may have different mechanism of action in acute and chronic stress.

Based on attenuating effects of crude extract of *Centella asiatica* on stress induced enhanced adrenal gland weight; enhance plasma glucose, corticosterone, it may be concluded that crude extract of *Centella asiatica* may have potent adaptogenic/antistress activity. Further isolation and characterization of bioactive constituents of *Centella asiatica* is needed for their pharmacological study.

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