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Chicoric acid regulates behavioral and biochemical alterations induced by chronic stress in experimental Swiss albino mice

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ABSTRACT

The present study was taken up to see the effect of chicoric acid (CA) on behavioral and biochemical alterations induced by chronic restraint stress in experimental Swiss albino mice. CA at 1 mg/kg dose level exhibited considerable antidepressant activity as shown by significant decrease in immobility period in the Porsolt's swim stress-induced behavioral despair test and escape failures in Learned "helplessness test". The antidepressant activity shown by CA can be attributed to its modulating effect on nor-adrenaline (NA), dopamine (DA) and 5- hydroxy tryptamine (5-HT) as shown by their quantification in CA treated chronically stressed mice. Further, a significant antioxidant effect was exhibited by CA as shown by estimation of lipid peroxidation, glutathione (GSH) and glycogen in liver of chronically stressed mice. It also normalized altered values of serum glucose, triglycerides, aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in a dose dependent manner. The stress busting potential of CA was further confirmed by its regulating effect on raised plasma corticosterone levels and significant attenuation of the depleted ascorbic acid, cholesterol and corticosterone levels in adrenal glands. Thus, our results suggest that CA possesses considerable stress busting potential, and that anti-oxidation may be one of the mechanisms underlying its antistress action.

1. Introduction

Stress is a global menace fortified by the advancement of industrialization and elicited by a variety of factors, viz., environmental, social or pathological phenomenon of life. Considerable evidences published in the last decade have focused on a constellation of neurochemical, biochemical, and molecular effects caused by stress in the CNS, endocrine system, and immune system (Aloe et al., 2002). Normally stress-induced changes are self limiting and adaptive until and unless events that override "threshold" limits become irreversible and pathological (McCarty, 1987). When stress becomes chronic, the HPA axis and other neuroendocrine pathways may be activated continuously and cause prolonged activation of enhanced catabolism leading to exhaustion of the body's reserves (Korte et al., 2005; Dhabhar and McEwen, 2001; McEwen, 2004). Stress impairs antioxidant defenses, leading to oxidative damage, by changing the balance between oxidant and antioxidant factors which contributes to the occurrence of many pathological conditions (Liu et al., 1994; Sosnovsky and Kozlov, 1992). In addition, chronic stress may cause behavioral changes, including depression, increased anxiety, fatigue and memory loss (Quervain, et al., 1998; Strekalova, et al., 2005). It also increases the risk for many health disorders, including coronary heart disease, hypertension, eating disorders, ulcers, diabetes, asthma, depression, migraine headaches, sleep disorders, chronic fatigue, and certain types of cancer.

Recently, medicines of herbal origin have gained a significant chunk of followers because of their acclaimed safety associated with prolonged treatment. Chicoric acid (CA) occurs naturally in large number of medicinal plants that are being used in folk medicine since time immemorial. *Taraxacum officinale* Weber, is one of the such plants which is locally known as "dandelion". Its anti-angiogenic, anti-inflammatory and anti-nociceptive activities are well documented (Jeon et al., 2008). This plant has long been used in folk medicine to treat hepatic disorders and some women's diseases, such as breast and uterus cancers, and as lactating, choleretic, diuretic, and anti-inflammatory remedies (Ahmad et al., 2000; Kisiel and Barszcz, 2000). Chicoric acid is one of the main constituents of *Taraxacum officinale*. It is also known as dicaffeyltartaric acid and belongs to phenylpropanoids (Zaprometov, 1993). This group of compounds is of special interest because of their high biologic activity (Kurkin, 1996). Chicoric acid is well documented as potent inhibitor of the Type 1 human immunodeficiency virus (HIV-1) integrase which catalyses the integration of HIV DNA copy into the host cell DNA (Robinson et al., 1996). Recently, Tousch D and co-workers from University

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Montpellier, France reported its effects on glucose uptake and insulin secretion in cell studies. It is considered as an active candidate for diabetes (Tousch et al., 2008) and it has also been shown to have a stimulatory effect upon phagocytes (Bone, 1998) and also exhibits antioxidant activities (Dalby-Brown et al., 2005).

In the present paper, 14 days chronic immobilization stress was applied to mice to induce biochemical and behavioral alterations and the ability of CA, obtained from *Taraxacum officinale* to reinstate these alterations was studied.

2. Materials and methods

2.1. Plant material

For isolation of Chicoric acid, *Taraxacum officinale* aqueous extract (aerial part) was clarified by ultrafilteration through a PLAC cellulose acetate filter (Millipore, USA), acidified with 0.1% HCl and concentrated with C18 cartridges (Waters, USA). The concentrated and purified extract (5 ml) was loaded on LH-20 sephadex coloumn (2×90 cm, pharmacia, Sweden) in water to acetic acid solution of 10:1 v/v and washed with same solution. Chicoric acid was eluted with $CHCl_3$ at a rate of 1.5 ml/min in 5 ml fractions. The fraction was vaccum concentrated at 40 °C to 1/15th of the initial volume and cooled yielding colorless filamentous crystals. The crystals were dissolved in water and lyophilized. The yield of CA was 6.22%. The structure of CA is shown in Fig. 1A.

The purity of the Chicoric acid was monitored by HPLC with UV detection. High-performance liquid chromatography was performed in a Waters chromatograph (USA) equipped with a Waters 600E pump and a computer-operated 990 UV detector. Samples were chromatographed on a 3.9×150 mm Novopak C-18 column (5-m pore size; 250×4.0-mm internal diameter) and eluted with acetonitrile – water, 60:40 v/v. The gradient of eluent was 0 to 50% in 25 min at a flow of 1.5 ml/min (Fig. 1B). The purity of chicoric acid was found to be more than 95%.

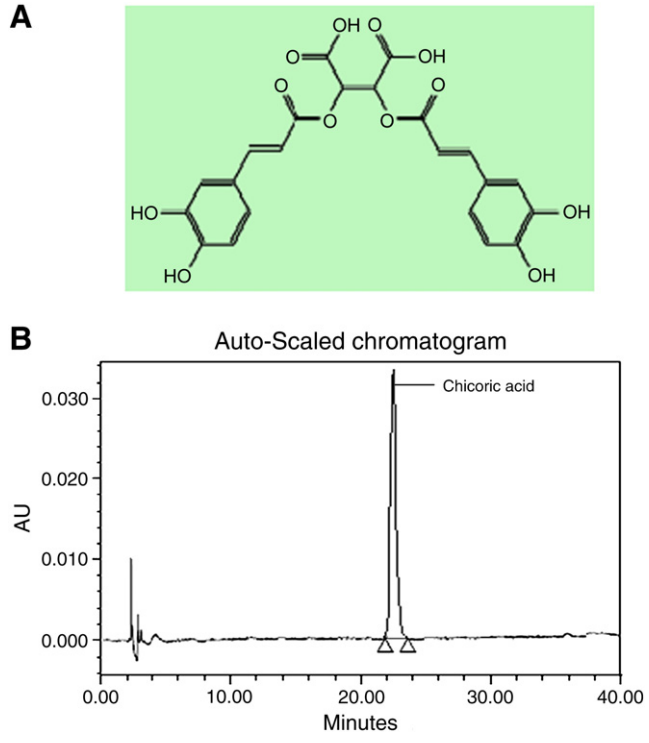

Fig. 1. A. Structure of Chicoric acid (CA). B. HPLC of Chicoric acid.

2.2. Restraint stress protocol

Male Swiss albino mice, 10–12 weeks old and weighing about 22–24 grams were employed for the study. All the animals were kept in isolators and were given free access to pellet food and water. The animals used for the experimental work were duly approved by our Institutional Animals Ethics Committee (IAEC) after verifying the protocols that were followed for carrying out the experiments. According to ethical regulations on animal research, all the animals used in the experimental work received proper humane care. Polypropylene tubes (50 ml), having proper ventilation, were used to induce stress to the animals during the experimental period. Mice were restrained in these 50 ml conical polypropylene tubes for 12 h during the dark cycle (2000–0800 h) for 14 days (Kour et al., 2009). Diagrammatic representation of the procedure adopted in this study is shown in the Fig. 2.

Following group configurations were used: Group 1 served as normal control without any restraint stress as well as test drug, group −2 was restraint stress control (RSC) group, and group 3 was administered CA *per se* at 2 mg/kg with no stress conditions. Group 4, 5, 6 and 7 were treated groups wherein CA was administered at the dose range of 0.25, 0.5, 1 and 2 mg/kg, p.o, respectively along with restraint stress conditions. Each group consists of six mice (n=6). Drug was administered once daily for 14 days, concomitantly to restraint stress procedure, two hours before subjecting the mice to restraint stress.

2.3. Behavioral parameters

Chronic stress is known to induce endogenous depression. The following methods were used to assess depressive behavior in restrained stress induced chronically stressed mice.

2.3.1. Swim stress induced "behavioral despair

On day 14 after final stress exposure, mice were made to swim individually in a polypropylene vessel (45×40×30 cm) with a water level of 20 cm. This ensured that the animals' feet did not touch the floor of the vessel and that it could not climb out of it. Each mouse was allowed to swim for 10 min. Thereafter, during the next 5 min, the periods of total immobility, characterized by complete cessation of swimming with the head floating just above water level, was noted. This immobility period, after the initial frenzied attempts to escape, is postulated to represent behavioral despair as an experimental model of endogenous depression (Porsolt et al., 1978).

2.3.2. Learned "helplessness test"

On Day 12 of the investigation, mice were subjected to footshock (60 scrambled shocks, 15 s duration, 0.8 mA, every minute) in a two-compartment jumping box (Techno) with the escape door to the un electrified adjoining compartment closed. The exercise continued for 1 h. On Day 14, 48 h afterwards the mice were subjected to avoidance training, using the same apparatus but keeping the escape route to the unelectrified chamber open. During this avoidance training, the mice were placed in the electrified chamber and allowed to acclimatize for 5 min before being subjected to 30 avoidance trials, with an inter-trial interval of 30 s. During the first 3 s of the trial, a buzzer stimulus (conditioned stimulus) was presented, followed by electroshock (unconditioned stimulus) (0.8 mA) through the grid floor for the next 3 s. The avoidance response, characterized by escape to the adjoining "safe" chamber during conditioned stimulus, was noted. Failure to escape during unconditioned stimulus within 15 s was assessed as "escape failure," which is postulated to indicate depression (Thiebot et al., 1992).

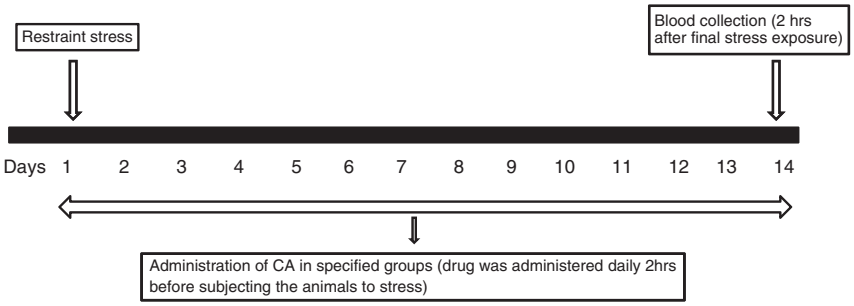

Fig. 2. Diagrammatic representation of the methodology adopted in the study.

2.4. Biochemical parameters

Estimation of biochemical parameters was carried out in brain region, liver, serum and adrenal glands following behavioral assessment on day 14.

2.4.1. Estimation of neurotransmitters in brain region

During stressful conditions, changes in monoamines (NA, DA and 5-HT) are well associated with transient behavioral aberrations in memory learning and other mood disorders. Levels of NA, DA and 5-HT were estimated in whole brain region of chronically stressed mice using High Performance Liquid Chromatography with Electrochemical detector as described by Kim et al. (1987). In brief, the brain tissue samples were homogenized in 0.17 M perchloric acid by Glass homogenizer. Homogenates were then centrifuged at 33,000×*g* (Biofuge Stratos, Heaureas, Germany) at 4 °C. Twenty microliter of supernatant was injected via HPLC pump (Model 1525, Binary Gradient Pump, Waters, Milford, MA, USA) into a column (Spherisorb, RP C18, 5 μm particle size, 4.6 mm i.d.×250 mm at 30 °C) connected to a Electrochemical detector (Model 2465, Waters, Milford, MA, USA) at a potential of +0.8Vwith glassy carbon working electrode Vs Ag/AgCl reference electrode. Mobile phase consists of 32mMcitric acid, 12.5 mM disodium hydrogen orthophosphate, 1.4 mM sodium octyl sulfonate, 0.05 mM EDTA and 16% (v/v) methanol (pH 4.2) at a flow rate of 1.2 mL/min.

2.4.2. Estimation of corticosterone

Immediately after the last stress regimen, animals were sacrificed by decapitation and blood was collected in EDTA coated tubes kept in ice and centrifuged at 1000×*g* for 20 min at 4 °C. The corticosterone assay was performed by competitive immunoenzymatic method (Elisa kit Neogen Corporation, USA). All samples were assayed in triplicates at a wavelength of 450 nm.

2.4.3. Estimation of biochemical parameters in adrenal glands

On day 14, after final stress exposure, the animals of all the groups were sacrificed; their adrenal glands were removed and weighed (Selye, 1936). The corticosterone (Zenker and Bernstein, 1958), ascorbic acid (Roe and Kuether, 1949) and cholesterol (Zlatkis et al., 1953) in adrenal glands were estimated after homogenization in sterile cold normal saline.

2.4.4. Estimation of hepatic parameters

The livers of all the animals were quickly excised, cleaned of adhering tissue weight and homogenized in chilled phosphate buffer saline in a potter-S-homogenizer (B. Braun, Germany) consisting of a Teflon pestle and glass homogenizer. The homogenization, and centrifugation, etc. was carried out at 0–4 °C. The supernatant was used for the assay of Lipid peroxidation (MDA), glutathione (GSH) and glycogen. The hepatic lipid peroxidation was measured by estimating malondialdehyde (MDA) as described by Buege and Aust (1978). The hepatic glutathione was determined by the methods of Ellman (1959), David et al. (1987) and hepatic glycogen was estimated using the method as given by (Hawk et al., 1978).

2.4.5. Estimation of biochemical parameters in serum

For the estimation of triglycerides, glucose, AST, ALT and ALP in serum, blood was collected from control as well treated groups before sacrificing them for biochemical analysis of adrenal glands and liver. Serum was obtained by centrifuging the blood at 5×g rpm for 10 min at 4 °C. Serum was stored at -80 °C until analysis. Glucose, triglycerides, aspartate aminotransferase (AST) alanine aminotransferase(ALT) and alkaline phosphatase (ALP) was estimated in serum using universal auto analyser (RAYTO, RT 1904-c) with their respective kits (CPC diagnostics, Riachem, USA) according to manufacturer's instructions (Kour et al., 2009).

2.4.5.1. Statistical analysis. Data is expressed as Mean ± S.E.M. Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons).

2.5. Results

2.5.1. Swim stress induced "behavioral despair"

Chronic stress induced significant increase in the immobility period in the Porsolt's swim stress-induced behavioral despair test ($p<0.001$). This increase was substantially reversed by treatment of animals with graded doses of CA. The most significant effect was observed at 1 and 2 mg/kg ($p<0.001$ (Fig. 3).

2.5.2. Learned "helplessness test"

Likewise, Chronic stress induced significant increase in escape failures, concomitant with decrease in avoidance responses. Treatment

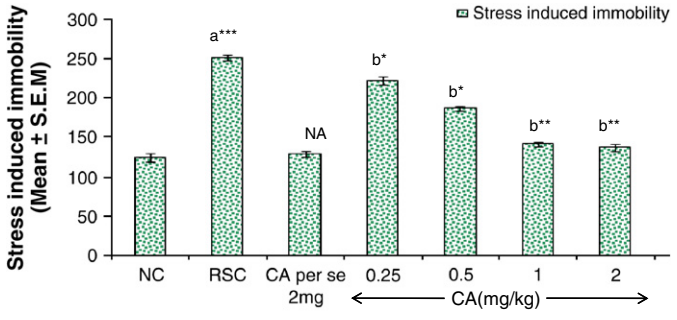

Fig. 3. Effect of graded doses of CA (mg/kg) on Porsolt's swim stress-induced behavioral despair test in chronically restrained mice. Chronic stress was induced by immobilizing the mice for 14 days. NC- normal control; RSC- restraint stress control; CA- chicoric acid. Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons). *P-values.* $^{*}p\leq0.05$, $^{**}p\leq0.01$,$^{***}p\leq0.001$. Asterisks with *p* value 'a' indicate significant difference of RSC *vs*.NC control and 'b' indicate CA treated groups *vs* RSC group.

Table 1
Effect of CA (mg/kg) on chronic stress induced increase in "learned helplessness" in mice.

S. No.	Treatment	Dose (mg/kg)	Escape failure *(n)*	Avoidance response *(n)*
1.	NC	-	15.61 ± 0.90	6.23 ± 0.70
2.	RSC	-	28.43 ± 1.10 (82.12↑)^{a***}	1.84 ± 0.47 (70.46↓) ^{a***}
3.	CA *per se*	2	15.02 ± 0.72 (3.77↓)	6.12 ± 0.53 (1.76↑)
4.	CA	0.25	24.20 ± 0.83 (14.87↓)^{bns}	2.90 ± 0.64 (57.60↑) ^{b**}
5.	CA	0.50	18.45 ± 0.56 (35.10↓) ^{b**}	3.79 ± 0.75 (105.97↑) ^{b***}
6.	CA	1.0	16.21 ± 0.57 (42.98↓) ^{b**}	4.26 ± 0.69 (130.43↑) ^{b***}
7	CA	2.0	15.45 ± 0.89 (45.65↓) ^{b**}	3.64 ± 0.52 (97.82↑) ^{b***}

NC- normal control, RSC- restraint stress control, CA- chicoric acid. Data is represented as Mean ± S.E.M (n = 6).
Test drug was administered once daily for the duration of the experiment 1 hr before subjecting the animals to stress.

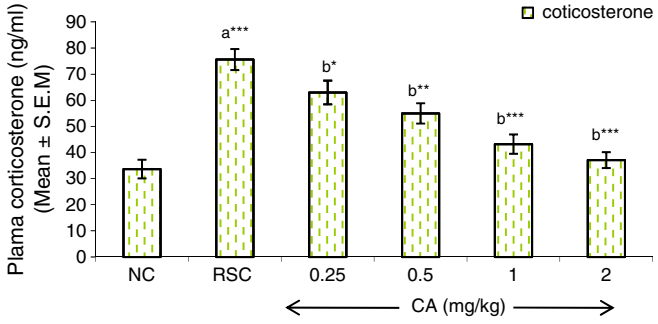

Fig. 4. Data shows the effect of CA on raised corticosterone levels in the plasma of chronically stressed mice on day 14. NC- normal control; RSC- restraint stress control; CA- chicoric acid. Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons). *P-values.* $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. Asterisks with *p* value 'a' indicate significant difference of RSC *vs*.NC control and 'b' indicate CA treated groups *vs* RSC group.

with CA, its parent extract TO-10 (100 mg/kg) ($p<0.01$) and parent fraction C.F (10 mg/kg) ($p<0.01$) reversed these effects. In case of CA, most considerable effect was observed at the dose level of 1 and 2 mg/kg ($p<0.001$) (Table 1).

2.5.3. Corticosterone assay

Corticosterone is the main marker of stress response in rodents. Its concentration increased in mice subjected to restraint stress (RSC-control) at least three times as compared to the Normal control group. However, CA exhibited a significant normalizing effect on the level of corticosterone with significant effect observed at the dose level 1and 2 mg/ kg, where it was 57.40 ± 3.25 and 50.88 ± 2.24 ng/ mL respectively (Fig. 4).

2.5.4. Estimation of neurotransmitters in brain region

Neurotransmitters, nor-adrenaline (NA), dopamine (DA) and 5-hydroxy tryptamine (5-HT) are the important monoamines which are widely distributed in brain and their functional role is well established during stressful conditions (Tsigos and Chrousos, 2002; Gonzalo et al., 2003). Determination of NA, DA and 5-HT levels by High Performance Liquid Chromatography revealed that chronic stress causes a significant depletion of these neurotransmitters ($p<0.001$) thus leading to the state of depression. Nevertheless, treatment with CA, attenuated the levels of NA ($p<0.01$), DA ($p<0.05$) and 5-HT ($p<0.01$) ($p<0.05$) at the dose levels of 1 and 2 mg/kg (Fig. 5).

2.5.5. Effect of CA on biochemical parameters in adrenal glands

Exposure to chronic restrain stress causes hypertrophy of the adrenal glands which is associated with significant depletion of adrenal contents of ascorbic acid, corticosterone and cholesterol ($p<0.001$). Treatment of animals with CA significantly attenuated the restraint stress induced hypertrophy of adrenal glands and depletion of adrenal ascorbic acid, cholesterol and corticosterone levels ($p<0.001$). The most significant effect was observed at the dose level of 1 mg/kg (Table 2).

2.5.6. Effect of CA on hepatic parameters

Chronic restraint stress also caused a significant alteration in hepatic parameters as evident from increase in Lipid peroxidation and decrease in glycogen and glutathione content. Treatment with CA significantly restored the altered values in a dose dependent manner. However, the most significant effect was obtained at the dose level of 1 and 2 mg/kg (Table 3).

2.5.7. Effect of CA on serum parameters

A significant increase in serum levels of Alanine transaminase (ALT), Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and glucose and a decrease in the levels of triglycerides was observed in animals subjected to chronic restraint stress ($p<0.001$). CA significantly

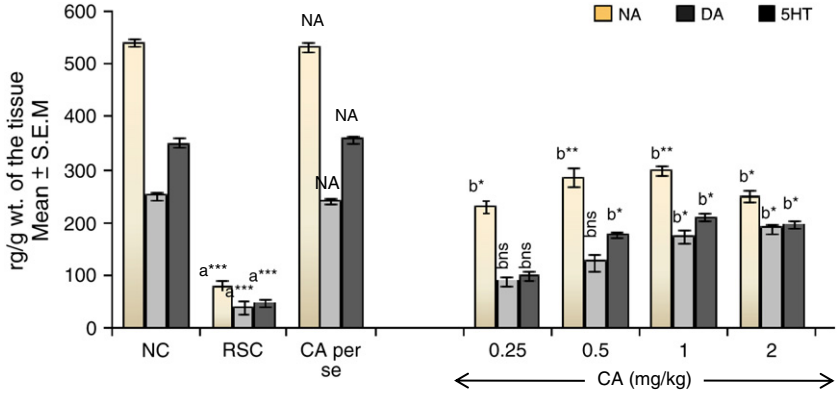

Fig. 5. Bar graphs represent the effect of CA on neurotransmitters, nor-adrenaline (NA), dopamine (DA) and 5- hydroxy tryptamine (5-HT) levels These were quantified on day 14 in whole brain region of chronically stressed mice using High Performance Liquid Chromatography with Electrochemical detector. NC- normal control; RSC- restraint stress control; CA- chicoric acid. Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons). *P-values.* $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. Asterisks with *p* value 'a' indicate significant difference of RSC *vs*.NC control and 'b' indicates CA treated groups *vs* RSC group.

Table 2
Effect of CA (mg/kg) on biochemical alterations induced by stress in adrenal glands.

S. No.	Treatment	Dose (mg/kg)	Ascorbic acid (mg/kg of ad. wt)	Cholesterol (g/100 g of adrenal. wt.)	Corticosterone (μg/100 g of adrenal wt.)	Adrenal wt. (mg/100 mg of live wt.)
1.	NC	-	342.38 ± 4.81	4.11 ± 0.55	5.11 ± 0.22	14.22 ± 0.64
2.	RSC	-	172.41 ± 5.02^{a**} (49.64↓)	1.96 ± 0.2 ^{a**} (52.31↓)	1.43 ± 0.08 ^{a**} (72.01↓)	38.62 ± 2.99 ^{b***} (171.58↑)
3.	CA *per se*	2	± 4.81	4.09 ± 0.15	5.00 ± 0.25	14.11 ± 1.06
4.	CA	0.25	295.15 ± 4.02^{b**} (71.19↑)	3.29 ± 0.23^{b**} 67.85↑	1.92 ± 0.21 ^{b*} (34.26↑)	28.16 ± 1.43 ^{b*} (27.08↓)
5.	CA	0.50	319.41 ± 3.96 ^{b**} (85.26↑)	3.42 ± 0.25 ^{b**} (74.48↑)	2.46 ± 0.27 ^{b**} (72.02↑)	24.00 ± 0.86 ^{b*} (37.85↓)
6.	CA	1.0	331.48 ± 4.25 ^{b***} (92.26↑)	3.70 ± 0.11 ^{b***} (88.77↑)	3.43 ± 0.23 ^{b***} (139.86↑)	19.23 ± 0.96 ^{b**} (50.20↓)
7	CA	2.0	329.27 ± 4.93 ^{b***} (90.75↑)	3.63 ± 0.08 ^{b***} (85.29↑)	4.15 ± 0.25 ^{b***} (190.20↑)	17.92 ± 2.05 ^{b**} (53.59↓)

↑: Increase; ↓: Decrease.
Value in parenthesis represents percent activity.
NC- normal control; RSC- restraint stress control; CA- chicoric acid. Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons). *P-values.* $^{*}p \leq 0.05$, $^{**}p \leq 0.01$,$^{***}p \leq 0.001$. Asterisks with *p* value 'a' indicate significant difference of RSC *vs*. NC control and 'b' indicate CA treated groups *vs* RSC group.

reinstated the altered values in a dose dependent manner, the most significant effect was observed at 1 mg/kg (Table 4).

3. Discussion

Stress as a health problem is on the rise and so are the stress relief products. Though there is a long list of drugs used in the treatment of depression, including selective serotonin reuptake inhibitors (SSRIs), a typical antidepressants, tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs), the list of their side effects like sexual problems, drowsiness, sleep difficulties, nausea headaches, back ache, neck pain, frozen shoulders, and other ailments is equally long. While some side effects go away after the first few weeks of drug treatment, others persist and may even get worse. So, everyone is on the lookout for natural stress relief techniques which are very effective and at the same time don't have many side effects. Present study shows CA obtained from *Taraxacum officinale* to possess significant stress busting activity by restoring the behavioral and biochemical alterations induced by chronic stress. Exposure to adverse situations affects an important number of aspects of our daily life. While response to stress is a necessary survival mechanism, prolonged stress can have several repercussions affecting behavioral, endocrine and immunological parameters (McEwen, 1998). Generalized stress, particularly if continued in nature, is known to induce melancholic depression (Gold et al., 1988). It has been suggested that the symptoms of endogenous depression represent tachyphylaxis of the mesocortical system to chronic activation of the stress system (Chrousos and Gold, 1992). The experimental paradigms used in this investigation to induce behavioural states akin to clinical depression have been subjected to extensive scrutiny and validated for inducing behavioral despair and for the evaluation of putative antidepressants (Thiebot et al., 1992). Our results clearly indicate that exposure of animals to chronic restraint stress for 14 days exhibited significant depressive behavior as shown by decrease in immobility period in the Porsolt's swim stress-induced behavioral despair test and escape failures in Learned "helplessness test" These effects were significantly reversed by treatment with the graded doses of CA. The most significant effect was however, obtained at 1 mg/kg dose level.

Table 3
Data shows the effect of graded doses of CA (mg/kg) on stress induced alterations in hepatic parameters.

S. No.	Treatment	Dose (mg/kg)	Hepatic Parameters		
			Glycogen μmol of p-nitrophenol formed/min/l	Lipid peroxidation mg/g liver	Glutathione nmol GSH/gliver
1.	NC	-	5.06 ± 0.56	30.25 ± 1.36	7.62 ± 0.91
2.	RSC	-	0.96 ± 0.11^{a***} (81.02↓)	61.31 ± 2.21 ^{a***} (102.67↑)	3.00 ± 0.23^{a***} (60.62↓)
3.	CA *per se*	2	5.12 ± 0.42	31.21 ± 1.05	7.59 ± 0.19
4.	CA	0.25	2.72 ± 0.15^{b*} (183.33↑)	50.46 ± 1.85^{b*} (17.69↓)	4.38 ± 0.33^{b*} (46.00↑)
5.	CA	0.5	3.56 ± 0.11^{b**} (270.83↑)	44.29 ± 1.98^{b**} (27.76↓)	5.61 ± 0.22^{b*} (87.00↑)
6.	CA	1.0	4.31 ± 0.09^{b***} (348.95↑)	36.39 ± 0.86^{b**} (40.64↓)	6.72 ± 0.17^{b**} (124.00↑)
7	CA	2.0	4.49 ± 0.13^{b***} (367.70↑)	32.10 ± 1.02^{b***} (47.64↓)	6.41 ± 0.21^{b**} (113.66↑)

↑: Increase, ↓: Decrease.
NC- normal control; RSC- restraint stress control; CA- chicoric acid.
Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons). *P-values.* $^{*}p \leq 0.05$, $^{**}p \leq 0.01$,$^{***}p \leq 0.001$. Asterisks with *p* value 'a' indicate significant difference of RSC *vs*.NC control and 'b' indicate CA treated groups *vs* RSC group.

It has also been postulated that cognitive dysfunction and behavioral depression, induced by stress, may be induced by similar neurochemical mechanisms, including depletion of monoamines by sustained stress (Anisman et al., 1998; Bhattacharya et al., 2002). Out of various neurotransmitters nor-adrenaline (NA), dopamine (DA) and 5- hydroxy tryptamine (5-HT) are the important monoamines which are widely distributed in brain and their functional role is well established during stressful conditions (Tsigos and Chrousos, 2002; Gonzalo et al., 2003). A significant alteration in the levels of these monoamines was observed in case of animals subjected to chronic restraint stress for 14 days. Dysfunction of these monoamines due to prolonged stressful conditions has been associated with a wide range of central and peripheral disorders like depression, and anxiety (Jayanthi and Ramamoorthy, 2005; Filip et al., 2005). CA at 1 mg/kg dose level exhibited considerable reversal effect on the altered values of nor-adrenaline (NA), dopamine (DA) and 5- hydroxy tryptamine (5-HT) as shown by their quantification in CA treated chronically stressed mice. The stress busting potential of CA can be further attributed to its normalizing effect on corticosterone levels which is the main marker of stress response in rodents and is otherwise elevated in chronic stressful conditions.

It has been shown that exposure to stress situations can stimulate numerous pathways leading to increased production of free radicals (Liu et al., 1996; Liu and Mori, 1999). It is well known that free radicals generate a cascade, producing lipid per oxidation, protein oxidation, DNA damage and cell death, and contribute to the occurrence of pathological conditions. This also results in progressive deterioration in most of the endocrine functions and enhances exogenous or endogenous stress associated with an impairment of hepato- pancreatic

Table 4
Effect of graded doses of CA on biochemical alterations induced by chronic stress in serum.

S. No.	Treatment	Dose	Triglycerides	Glucose	AST	ALT	ALP
		(mg/kg)	mg/dL	mg/dL	IU/L	IU/L	IU/L
1.	NC	-	86.45 ± 4.15	74.38 ± 5.09	126.12 ± 6.01	48.65 ± 3.01	23.67 ± 2.49
2.	RSC	-	50.29 ± 5.56^{a**} (41.82↓)	132.78 ± 5.78^{a***} (78.51↑)	197.02 ± 7.23^{a**} (56.21↑)	71.34 ± 4.98^{a**} (46.63↑)	42.78 ± 3.55 ^{a***} (80.73↑)
3.	CA *per se*	2	83.56 ± 3.24	76.67 ± 4.98	122.67 ± 8.09	43.38 ± 3.66	20.56 ± 3.09
4.	CA	0.25	55.67 ± 4.98^{bns} (10.69↑)	120.42 ± 5.65 ^{bns} (9.30↓)	172.56 ± 7.55 ^{bns} (12.41↓)	72.77 ± 4.56 ^{bns} (2.00↓)	37.82 ± 4.68^{bns} (11.59↓)
5.	CA	0.5	62.59 ± 6.54 ^{b*} (24.45↑)	100.40 ± 7.23 ^{b*} (24.38↓)	157.45 ± 4.89 ^{b*} (20.08↓)	65.78 ± 4.31^{bns} (7.79↓)	32.46 ± 5.23^{b*} (24.12↓)
6.	CA	1.0	68.15 ± 4.76 ^{b**} (35.51↑)	89.54 ± 6.97 ^{b**} (32.56↓)	141.28 ± 4.12^{b**} (28.29↓)	58.39 ± 3.43^{b*} (18.15↓)	27.45 ± 4.33^{b**} (32.83↓)
7	CA	2.0	73.39 ± 5.97 ^{b**} (45.93↑)	93.41 ± 8.79 ^{b**} (29.65↓)	138.21 ± 5.89^{b**} (29.84↓)	54.34 ± 3.78 ^{b*} (23.82↓)	24.51 ± 4.98 ^{b**} (42.70↓)

↓: Increase; ↓: Decrease.
Value in parenthesis represents percent activity against control. Statistical significance of differences was assessed by Post-ANOVA (Bonferroni test for multiple comparisons). *P-values.* $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.
Asterisks with *p* value 'a' indicate significant difference of RSC *vs*. NC and 'b' indicate CA treated groups *vs* RSC group.

function (Kumar et al., 1992; Zbigniew, 1994), and in turn causes a significant elevation of the activities of GPT, GOT, ALP, bilirubin triglycerides in serum and liver glycogen, lipid-per oxidation and GSH, indicating considerable alteration in normal physiological functioning of the body.GSH is the most important endogenous protective biomolecule against adverse conditions. In this connection, the protective role of GSH against cellular lipid per-oxidation has been well documented (Burk, 1983). A substantial increase in hepatic lipid per-oxidation is evident from elevated MDA levels, which is an end product of lipid per oxidation and a good indicator of oxidative injury in liver homogenate with a concurrent fall in hepatic GSH and glycogen contents following chemical and physical stress is indicative that stress alters the physiological functioning of the body. The increased MDA and reduced GSH and glycogen in the livers of the mice were significantly attenuated by treatment with CA at graded oral doses. A significant increase in serum levels of ALT, ALP, AST and glucose and a decrease in the levels of triglycerides was observed in animals subjected to chronic restraint stress which were again significantly reinstated by treatment with CA.

In conclusion, our study demonstrates that Chicoric acid (CA) is a potent stress busting agent of herbal origin, possessing the ability to restore chronic restraint stress induced behavioral and biochemical alterations. CA can be of therapeutic value for various stress related disorders, and anti-oxidation along with its ability to regulate HPA axis may be one of the mechanisms underlying its antistress action.

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