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ORIGINAL PAPER

Nardostachys jatamansi Protects Against Cold Restraint Stress Induced Central Monoaminergic and Oxidative Changes in Rats

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Abstract Cold restraint stress (CRS) model exerts similar effect as physiological stress because it combines emotional stress (escape reaction) and physical stress (muscle work). It is well established that various responses to stress are regulated by sympathoadrenal system, brain monoaminergic systems and oxidative processes. *Nardostachys jatamansi* (NJE) is known to possess soothing and sedative action on the central nervous system. The present investigation was performed to explore the anti-stress activity of NJE on CRS model, through its effect on biochemical and neurochemical alterations. The rats were restrained in metallic chambers for 3 h at 4 °C was followed by sacrifice and assessment of stress related alterations. Hydro-ethanolic (30:70) extract of NJE was administrated orally at the doses of 200 and 500 mg/kg for 14 days and compared with vehicle control and *Panax ginseng* (100 mg/kg). Effects of NJE on CRS induced oxidative stress including reduced glutathione, glutathione peroxidase, glutathione reductase, glutathione-s-transferase were estimated. Dopamine, norepinephrine, serotonin and 5-hydroxy indole acetic acid were measured in the cerebral cortex, hippocampus and hypothalamus by HPLC electrochemical detector. NJE at both doses significantly inhibited CRS induced oxidative stress. It significantly mitigated CRS induced altered level of neurotransmitters in different brain regions. The study implied that NJE has the ability to provide protection against CRS induced oxidative stress and neurochemical alterations. Findings indicated that NJE revealed potent anti-stress effect implicating its therapeutic importance in stress-related disorders.

Keywords Antistress activity · Restraint stress · Oxidative stress · *Nardostachys jatamansi* · Neurotransmitters · *Panax ginseng*

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Introduction

Exposure to restraint stress affects numerous responses including autonomic, visceral, immunological [1] and neurobehavioral responses like anxiety, depression, anorexia etc. [2, 3]. It is one of the well accepted stressor used in experimental stress research, which elicits the purest form of psychological frustration and physiological stress accompanied with vigorous struggle to escape [4–6]. Hence in this study cold restraint stress model has been used.

Studies have reported that both reactive oxygen species and reactive nitrogen species have been implicated in pathophysiological conditions and the balance is very important during several disease states [7]. The brain is extremely susceptible to free radical damage because it possesses abundant lipid content and high oxygen consumption as also suffers from scarcity of antioxidant enzymes compared to other tissues [8, 9]. Studies have shown that CRS can lead to produce oxidative stress in brain [4, 10, 11].

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It has been reported that, CRS influences the turnovers of several neurotransmitters, along with hormonal and behavioural changes in several animal species including rats [12]. The monoaminergic system and the corticosteroid receptors interact to mediate several changes in the brain [13]. Restraint stress produced an increase in norepinephrine (NE) in rat brain and an enhanced turnover of NE. It also increased dopamine (DA) and cause oxidative damages to the dopamine neurons [14]. Studies suggested that stress can modulate serotonin (5-HT) level, rate of synthesis, release, uptake, and turnover in a variable manner with type of stressors and regional differences [15].

Modern psychotropic drugs used to counteract the stress have shown that they do not address the psychopathology of stress and also produce numerous adverse effects. Moreover there is no specific anti-stress drug in modern medicine, though anxiolytics are used to cope up the stress [16]. Therefore, it is worthwhile to search newer and more effective antistress drugs. In this scenario we selected an Indian medicinal plant, *Nardostachys jatamansi* (NJE), for exploring its effects on cold restraint stress induced alterations of biochemical and neurochemical functions.

Although NJE exerted a wide range of pharmacological activities, including antiasthmatic, hypotensive, hypolipidemic, anti-ischemic, antiarrhythmic, cardioprotective, hepatoprotective, anticonvulsant, antiparkinsonian, antidiabetic, antipancreatitis. Nardostachysin, Nardosinone, Jatamansin, Jatamoles A and Jatamoles B have been isolated from this plant [17–25]. Our earlier study has shown that NJE has important role in preventing the adverse effects against CRS induced adrenocortical activation, gastric ulceration and oxidative stress [4]. It also protects from the chronic fatigue syndrome induced severe depression through altering oxidative stress [26]. However, no studies so far have dealt with the effects of NJE on cold restraint stress (CRS) mediated biochemical and neurochemical alterations till date. The mechanism of activity of NJE still remains unexplored.

Therefore the present investigation was aimed to study the CRS induced oxidative stress, neurochemical alterations, and to study NJE's putative mechanism of action as antistress agent. Efficacy of *Panax ginseng* as potent antistress and adaptogen had been demonstrated in several studies and was found effective on various psychiatric conditions [27–34]. Therefore, *Panax ginseng* has been used as a standard antistress drug.

Materials and Methods

Animals

Male albino Wister strain rats (130–160 gm) were used. Animals were procured from disease free animal house. The animals were housed properly in standard laboratory conditions with normal temperature 25 ± 2 °C and humidity between 40 and 60 % with alternate 12 h light and dark cycle. They had free access to food pellets (Vetcare Pvt. Ltd. Bangalore) and water according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA), Ministry of Social justice and Empowerment, Government of India, which complies with International norms of Indian National Science Academy [35]. The animals were maintained according to the guidelines of National Institute of Health ('Guide for the care and use of laboratory animals' NIH Publication No. 85–23). They had been acclimatized to laboratory conditions for 7 days before the study.

Preparation of Hydro-Ethanolic Plant Extract

The plant material was collected, identified and authenticated taxonomically by taxonomist of Botanical Survey of India, Indian Botanic Garden, Howrah. The voucher specimen of the collected sample (DB/ICMR//04) has been preserved at the departmental museum. The shade dried plant material, i.e. rhizomes of *Nardostachys jatamansi* was first coarsely powdered and extracted with 70 % ethanol at room temperature in a percolator. The extract was then concentrated in reduced temperature and pressure on rotary evaporator, followed by lyophilization and stored at 4 °C. The yield of dry extract from crude powder of jatamansi was 9 %. The chemical constituents of the extract were identified by qualitative group analysis and confirmed by thin layer chromatography. The amount of the active compound jatamansone in the extract was compared with jatamansone standard for standardization of the extract by HPTLC [36].

Drugs and Vehicle

The NJE extract was administered orally through gavage, once a day at two dose levels (200 and 500 mg/kg body weight). The doses were selected from the dose response trial and used in our previous studies [4, 26]. *Panax ginseng* (100 mg/kg body weight) has been used as standard anti-stress agent. The test and standard drug have been suspended in 0.3 % carboxymethyl cellulose (CMC). The drugs were administered for 14 days prior to induce restraint stress.

Experimental Design

Each experimental group comprised of 8 animals. Groups were as follows—I (Normal/Naive): Unstressed rats treated with vehicle only; II (Positive control): Jatamansi (NJE) treatment (500 mg/kg) only; III (Stress): stressed rats

treated with vehicle and serving as negative control; IV (Standard): stressed rats treated with *Panax ginseng* (100 mg/kg) and serving as standard control; V (T-200): stressed rats treated with NJE extract 200 mg/kg, and VI (T-500): stressed rats treated with NJE extract 500 mg/kg.

Cold Restraint Stress

On the last day of the drug treatment i.e., on the 14th day, after 1 h of the last dose, animals were subjected to stress by cold restraint or immobilization. Minimal handling was used to put the rats into the restraint chamber, consisting of a close-ended cylinder. Large breathing holes at the front end of the chamber provided adequate ventilation. The fore and hind limbs were tied separately and then together with adhesive tape. The rats were then individually inserted in restraint chamber; the tail was taped to the side to completely immobilize the animal then kept for 3 h at 4 °C in a refrigerator [4, 12]. The Institutional Animal Ethics Committee (CPCSEA/544) had approved the plan of animal protocol.

Cold Restraint Stress Induced Oxidative Stress

Preparation of Brain Homogenate

After completion of the stress schedule, rat was sacrificed through cervical dislocation, brain was removed immediately after sacrifice, washed with ice cold isotonic NaCl and dried properly. Both oxidative stress and neurotransmitters assay were carried out in the same animals. Hippocampus, hypothalamus and half of the cerebral cortex were dissected out for neurotransmitters assay, rest of the brain was used for the study of oxidative stress. Brain homogenate (10 % w/v) was prepared using phosphate buffer (pH-7.4) and centrifuged in 15,000 rpm for 20 min.

Determination of Reduced Glutathione (GSH)

Reduced glutathione was estimated according the method of Ellman et al. [37]. An equal volume of homogenate was mixed with 10 % TCA and centrifuged to separate the proteins. To 250 µl of this supernatant 2 ml phosphate buffer (pH 7.4), 250 µl DTNB (di-thio bis nitro benzoic acid) were added and mixed properly. The yellow colour developed was read at 412 nm. The result was calculated from the standard curve and expressed as µM/mg protein.

Determination of Glutathione Peroxidase (GPx) Activity

The reaction mixture consisted of 0.05 M phosphate buffer (pH 7.0), 1 mM EDTA, 1.4 U of 0.1 ml GR, 1 mM GSH, 0.2 mM NADPH, 0.25 mM H_2O_2, and 0.1 ml of homogenate in a final volume of 2.0 ml. Sodium azide (1.0 mM) was added to the reaction mixture in order to inhibit catalase activity according to the method of Bhattacharyya et al. [38]. The disappearance of NADPH at 340 nm was recorded. The enzyme activity was calculated as nM NADPH oxidized/min/mg protein.

Determination of Glutathione Reductase (GR) Activity

Glutathione reductase activity was measured as per Mohandas et al. [39]. The assay mixture consisted of 0.1 M phosphate buffer (pH 7.6), 0.1 mM NADPH, 0.5 mM EDTA, 1.0 mM oxidized glutathione (GSSG), and 0.1 ml of brain homogenate was added in a total volume of 3.0 ml. The enzyme activity was assessed by measuring the disappearance of NADPH at 340 nm. Result was expressed as nM NADPH oxidized/min/mg protein.

Determination of Glutathione-S-Transferase (GST) Activity

Glutathione-S-transferase (GST) activity was measured by the method of Athar et al. [40]. The reaction mixture contained 0.1 M phosphate buffer (pH 6.5), 1.0 mM reduced glutathione (GSH), 1.0 mM 1-chloro-2,4 dinitro benzene (CDNB), and 0.1 ml homogenate in a final volume of 2.0 ml. The changes in absorbance were recorded at 340 nm, and the enzyme activity was expressed as nM CDNB conjugate formed/min/mg protein.

Protein Estimation

The protein content was estimated according to Lowry et al. [41]. In brief, brain homogenate was mixed with Lowry solution (mixture of alkaline di-sodium carbonate, copper sulphate and sodium tartrate) and incubated for 15 min. Diluted Folin reagent was then added and read at 750 nm. The result was calculated from the standard curve and expressed as mg/ml.

Monoamines Assay in Rat Brain by HPLC

Brain regions including cerebral cortex, hypothalamus and hippocampus, were dissected out and weighed immediately. Samples were homogenized in ice cold 0.1 M HCl, on crushed ice, followed by centrifugation at 4000 rpm at 4 °C for 10 min. Supernatant was collected and filtered through 0.20 µm microfilters. Reverse phase HPLC analytical column (Nova pak C-18, 3.9 × 150 mm) coupled to an electrochemical detector (Waters Pulsed 464 EC Detector) was used for analysis. Concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and

its metabolite 5-Hydroxy-indole acetic acid (5-HIAA) were measured in three different regions of rat brain according to the method of Aydin et al. [42]. The mobile phase used consists of citric acid, sodium citrate, EDTA, heptasulphonic acid, glacial acetic acid, tetrahydrofuran and HPLC grade methanol. Volume was made up to 1 liter with HPLC grade water and pH adjusted to 4.9. Flow rate was set 1 ml/min with the potential set at 1200 PSI, with glassy carbon electrode vs Ag/AgCl reference electrode. 30 µl samples were injected via HPLC pump (Waters 515 HPLC pump) for detection. Individual neurotransmitters were identified by comparing their elution times with those of reference standards. The amount of each neurotransmitter was quantified from their respective peak heights obtained from the reference standards through the software adjusted with the system.

Results

Effect of NJE on Cold Restraint Stress Induced Oxidative Stress

Glutathione Level in Brain (GSH)

Analysis of result has shown that CRS decreased the glutathione level in the stress (CRS) group of animals ($P < 0.001$). After NJE treatment GSH level slightly increased in the non stressed animals ($P < 0.05$). While pre-treatment with test drug NJE (200 mg/kg) i.e., T-200 reversed the stress induced reduction of glutathione levels ($P < 0.01$). Similar with the above effect, T-500 group (NJE 500 mg/kg) significantly negated the depletion of GSH ($P < 0.001$). Standard group (STD) receiving PGE (100 mg/kg) also showed reversal effect ($P < 0.01$) against CRS induced alteration of glutathione level in brain (Table 1).

Glutathione Peroxidase (GPx)

The finding showed that CRS significantly inhibited glutathione peroxidase enzyme activity in the stress (CRS) group ($P < 0.001$). GPx enzyme activity was increased following the treatment of NJE alone to the unstressed animals ($P < 0.05$). Prior treatment with NJE (200 mg/kg) i.e., T-200 had elevated stress induced reduction of enzyme activity significantly ($P < 0.05$). In addition, T-500 (NJE 500 mg/kg) and standard (STD) group having PGE (100 mg/kg) also showed protective effects against CRS induced depletion of glutathione peroxidase enzyme in rat brain ($P < 0.001$ for both groups) (Table 1).

Glutathione Reductase (GR)

It has been observed that CRS significantly reduced glutathione reductase enzyme activity in the stress (CRS) group of animals ($P < 0.001$). The activity of GR was significantly increased by NJE treatment alone ($P < 0.05$). Where as T-200 ($P < 0.01$) and T-500 ($P < 0.001$) groups significantly attenuated the stress induced reduction of GR enzyme. Standard group with PGE (100 mg/kg) also negated GR activity when compared to stress group ($P < 0.001$) (Table 1).

Glutathione-s-Transferase (GST)

Exposure to CRS significantly reduced glutathione-s-transferase (GST) activity when compared to normal group of animals ($P < 0.001$). Administration of NJE alone to the unstressed animals significantly increased the GST activity in brain ($P < 0.05$). Treatment with NJE 200 mg/kg (T-200) ameliorated CRS induced reduction ($P < 0.01$). NJE (500 mg/kg) pretreated group (T-500) showed more significant reversal of stress induced depleted enzyme activity ($P < 0.001$). Standard drug treated group (STD) also showed similar effect ($P < 0.001$) (Table 1).

Table 1 Effect of NJE cold restraint stress induced oxidative stress in brain

	GSH (µM/mg protein)	GPx (nM/min/mg protein)	GR (nM/min/mg protein)	GST (nM/min/mg protein)
Normal	5.7 ± 0.3	37.7 ± 1.4	10.13 ± 0.16	31.8 ± 1
N. jatamansi	7.4 ± 0.5$^{\#}$	39.2 ± 1.8$^{\#}$	14 ± 1.1$^{\#}$	34.7 ± 1.7$^{\#}$
Stress (CRS)	3.1 ± 0.3$^{\#\#\#}$	26.2 ± 0.9$^{\#\#\#}$	8.03 ± 0.16$^{\#\#\#}$	18.9 ± 1.8$^{\#\#\#}$
STD + CRS	5 ± 0.1**	34.2 ± 0.3***	11.18 ± 0.3***	27.8 ± 0.6***
T-200 + CRS	5.2 ± 0.09**	31.5 ± 0.5*	10.46 ± 0.15**	24.5 ± 0.8**
T-500 + CRS	6.3 ± 0.1***	34.9 ± 0.6***	12.25 ± 0.5***	30 ± 0.4***

Values are represented as mean ± standard error of mean; n = 8 per group

Normal: unstressed; *N. jatamansi:* NJE alone (500 mg/kg); CRS: cold restraint stress; STD (standard): *Panax ginseng* extract 100 mg/kg; T-200: NJE 200 mg/kg + CRS and T-500: NJE 500 mg/kg + CRS

Statistical significance has been derived from ANOVA followed by post hoc test; $^{\#}$ $P < 0.05$; $^{\#}$ $P < 0.001$ compared to normal group; * denotes $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to stressed but untreated group

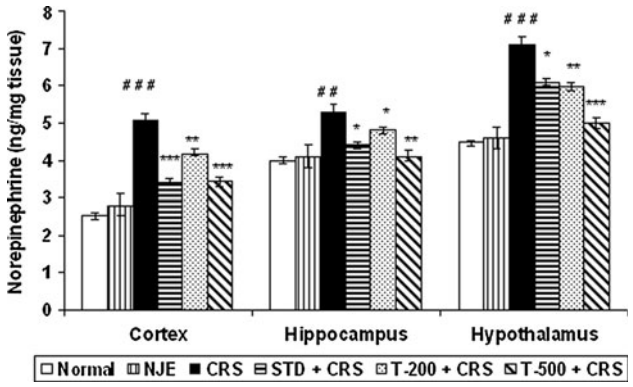

Fig. 1 Effect of NJE on CRS induced changes in norepinephrine level in different brain regions. Values are represented as Mean ± SEM; n = 8, Two way ANOVA followed by post hoc tukey's test analysis. # Represents when compared to Normal group $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. * Represents as compared to CRS group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Normal: normal unstressed, NJE: *N. jatamansi* alone (500 mg/kg), CRS: cold restraint stress, Standard: *P. ginseng* (100 mg/kg) + CRS, T-200 = *N. jatamansi* (200 mg/kg) + CRS, T-500 = *N. jatamansi* (500 mg/kg) + CRS

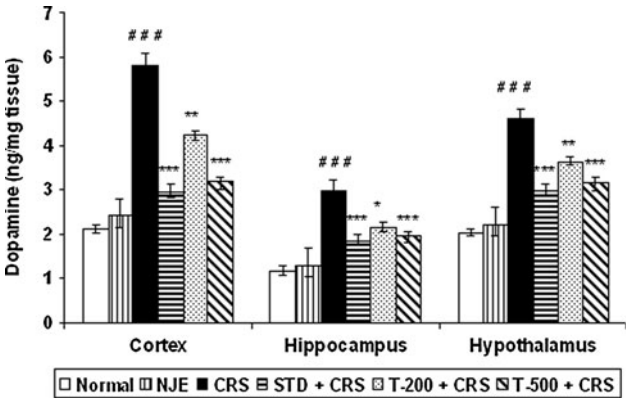

Fig. 2 Effect of NJE on CRS induced changes in dopamine level in different brain regions. Values are represented as Mean ± SEM; n = 8, Two way ANOVA followed by post hoc tukey's test analysis. # Represents when compared to Normal group $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. * Represents as compared to CRS group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Normal: normal unstressed, NJE: *N. jatamansi* alone (500 mg/kg), CRS: cold restraint stress, standard: *P. ginseng* (100 mg/kg) + CRS, T-200 = *N. jatamansi* (200 mg/kg) + CRS, T-500 = *N. jatamansi* (500 mg/kg) + CRS

CRS Induced Neurochemical Alterations

Norepinephrine Level

NE concentration was found to be augmented in cerebral cortex, hypothalamus ($P < 0.001$ for each region) and hippocampus ($P < 0.01$) after exposure to CRS. While NJE treatment *per se* did not produce any significant change in all the three brain regions. However, prior treatment with NJE at dose level of 200 mg/kg (T-200) significantly prevented the CRS induced augmentation of NE in cerebral cortex, hypothalamus ($P < 0.01$ in each region), and ($P < 0.05$) in hippocampus of rat brain. Similar effect was observed in T-500 group with NJE 500 mg/kg in cortex and hypothalamus ($P < 0.001$ in each region) and in hippocampus ($P < 0.01$). Standard drug PGE (STD) consistently reversed the CRS induced elevation of NE level in cortex ($P < 0.001$), hypothalamus and hippocampus ($P < 0.05$ in each region) (Fig. 1).

Dopamine Level

CRS had markedly increased the concentration of DA in brain regions like cortex, hippocampus and hypothalamus ($P < 0.001$ for each region). While NJE treatment *per se* insignificantly alters the DA level in all brain regions. Pretreatment with NJE at the dose of 200 mg/kg (T-200) significantly reduced the stress induced elevation of DA in the cortex, hypothalamus ($P < 0.01$ in each region) and in hippocampus ($P < 0.05$). In the same way 500 mg/kg dose of NJE (T-500) significantly decreased the elevation of DA in all the three brain regions ($P < 0.001$ in each region). Prior treatment with PGE-100 mg/kg (STD) significantly reversed the CRS induced rise of DA level ($P < 0.001$ in each region) (Fig. 2).

Serotonin Level

The observations revealed that 5-HT level was elevated in cortex and hypothalamus ($P < 0.001$ in each region) and in hippocampus ($P < 0.01$) after exposure to CRS. While NJE treatment *per se* showed no significant changes in all brain regions. Pretreatment with NJE (200 mg/kg) had mitigated the CRS induced elevation of 5-HT in cortex, hypothalamus and in hippocampus ($P < 0.01$ in each region). Consequently, NJE (500 mg/kg) treatment (T-500) significantly reversed the CRS induced elevation of 5-HT in all three regions more avidly ($P < 0.001$ in each region). PGE used as standard drug significantly prevented stress induced augmentation of 5-HT in cortex and hypothalamus ($P < 0.001$ in each region), and in hippocampus ($P < 0.05$) (Fig. 3).

5-Hydroxy-Indole Acetic Acid Level

5-Hydroxy-indole acetic acid (5-HIAA) the metabolite of 5-HT, was found to increase in cortex ($P < 0.001$), hippocampus ($P < 0.05$), and hypothalamus ($P < 0.001$) during cold restraint stress. While, NJE treatment alone was unable to produce any significant change in all the three brain regions. In T-200 group, significant change in 5-HIAA level was observed in cortex, hippocampus, and in hypothalamus ($P < 0.05$ for all regions) when compared to stress group. Treatment with NJE (500 mg/kg) significantly

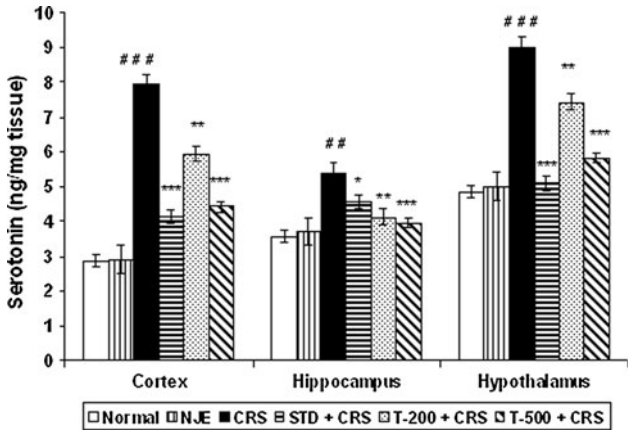

Fig. 3 Effect of NJE on CRS induced changes in serotonin level in different brain regions. Values are represented as Mean ± SEM; n = 8, Two way ANOVA followed by post hoc tukey's test analysis. # Represents when compared to Normal group $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. * Represents as compared to CRS group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Normal: normal unstressed, NJE: *N. jatamansi* alone (500 mg/kg), CRS: cold restraint stress, Standard: *P. ginseng* (100 mg/kg) + CRS, T-200 = *N. jatamansi* (200 mg/kg) + CRS, T-500 = *N. jatamansi* (500 mg/kg) + CRS

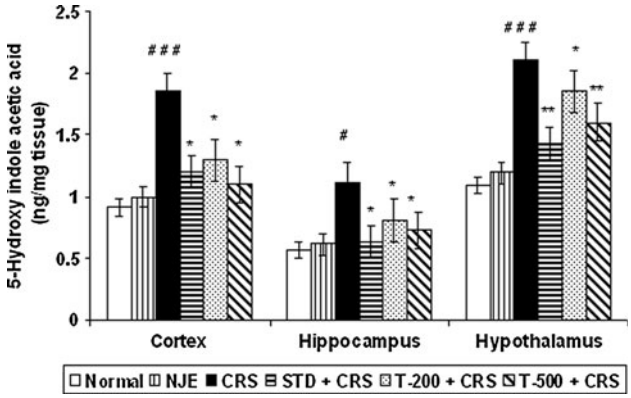

Fig. 4 Effect of NJE on CRS induced changes in 5-hydroxy-indole acetic acid (5-HIAA) level in different brain regions. Values are represented as Mean ± SEM; n = 8, Two way ANOVA followed by post hoc tukey's test analysis. # Represents when compared to Normal group $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. * Represents as compared to CRS group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Normal: normal unstressed, NJE: *N. jatamansi* alone (500 mg/kg), CRS: Cold restraint stress, Standard: *P. ginseng* (100 mg/kg) + CRS, T-200 = *N. jatamansi* (200 mg/kg) + CRS, T-500 = *N. jatamansi* (500 mg/kg) + CRS

modified the stress induced alterations in 5-HIAA in cortex, hippocampus ($P < 0.05$ in each region) and in hypothalamus ($P < 0.01$). PGE treatment (STD) exerted protective effect from stress induced alteration of 5-HIAA level in cortex and hippocampus ($P < 0.05$ for both regions), and in hypothalamus ($P < 0.01$) (Fig. 4).

Discussion

Cold restraint stress or forced immobilization stress model exerts similar effect as physiological stress because it combines emotional stress (escape reaction) and physical stress (muscle work), resulting in both restricted mobility and aggression. It is one of the effective and well explored models of stress in rats [43].

Cold restraint stress has been reported to induce oxidative stress in animals [44, 45]. CRS induced oxidative stress has been assessed by measuring the status of endogenous antioxidant system to ensure that whether the plant extract is able to cope up the CRS induced oxidative stress. Several antioxidant enzymes were evaluated to support the fact. The present result showed that exposure to CRS significantly decreased the non enzymatic antioxidant glutathione levels, and reduced the antioxidant enzyme activities in brain including glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-s-transferase (GST) significantly. Glutathione and these antioxidant enzymes play important role in antioxidant defense systems [46]. This study is in agreement with the previous observations [2, 47, 48]. However, NJE treatment attenuated stress-induced alteration of enzymatic and non enzymatic antioxidant system prominently. Moreover, NJE administration alone significantly increased the endogenous antioxidant level than normal. The results support the fact that presence of this antioxidant property would add to the pharmacological quality of NJE extracts. It has been reported that CRS up regulated the lipid peroxidation, nitric oxide production and simultaneously decreased antioxidant enzyme like catalase in rat brain as well as in stomach. Treatment with NJE reversed those stress induced effect, which provides consistency with its antioxidant properties [4]. Another observation has revealed that NJE possessed potent antioxidant effect as evidenced by effective free radical scavenging activity, and protective effect on hypoxia induced oxidative stress (Lyle et al., personal communication). A number of studies have shown that antioxidants both endogenous and dietary can protect nervous tissue from damage by oxidative stress [49]. Therefore this study revealed the fact that NJE exhibited anti stress effects at least partly due to its antioxidant effect. This result was corroborated with the previous studies [4, 10, 26].

It has been well accepted that acute stress is responsible to produce improved arousal, which influences the animal for adaptive response by means of avoidance. Arousal produces a complex phenomenon associated with changes in neurotransmitter levels including 5-HT, DA and NE in both humans and animals. It has been widely reported that changes in brain function mediated by stressors occur through alterations of neurotransmitter systems in the

brain. A number of centrally acting drugs exert their pharmacological actions through one or more of these putative neurotransmitters. CRS has been reported to activate monoaminergic system leading to enhanced monoamines like NE, DA and 5-HT in different regions of brain of the animals [50–53]. In consistent with the earlier study, the present study reported that in general CRS produced significant elevation of NE, DA, 5-HT and 5-HIAA levels in different brain regions including cortex, hippocampus and hypothalamus. In particular, NE was found to be increased in cerebral cortex, while NJE significantly reduced the higher NE level. It has been found that NE level was enhanced in hippocampus during CRS. An increased level of NE was also observed in hypothalamus during CRS exposure to rat. Hence, the elevation was higher in hypothalamus than cortex and hippocampus. A general activation of the norepinephrine neurons has been described in response to different stressors in rats. An enhanced turnover of NE metabolism was also described during stress in rats. Hence the present findings corroborated with the previous studies [54]. Test drug treatment significantly mitigated the effect of CRS in cortex, hippocampus and hypothalamus. The modulating effect of NJE on NE function rationalizes its antistress effects.

The current investigation also revealed that CRS significantly increased DA in cerebral cortex, hippocampus and the hypothalamus. Although, stress induced increment of DA in the hippocampus were found lower than cortex and hypothalamus. This result is also in accordance with the previous statements [55, 56]. Various types of stress such as mild electric foot shock, restraint, food deprivation, conditioned fear, all produce higher DA metabolism in the animals [57]. Treatment with NJE significantly (in the both doses) reversed the stress induced augmentation of DA in all three brain regions.

Serotonin and its metabolite 5-HIAA were measured in all three brain regions in order to study the 5-HT turn over, as it metabolized rapidly. The present study demonstrated that CRS significantly elevated 5-HT in cortex. Elevation of 5-HT in hippocampus also observed which was less than cortex and hypothalamus. In the hypothalamus, 5-HT level was found higher than other regions. It has been previously reported that immobilization or restraint stress was involved with up regulation of synthesis and metabolism of serotonin (5-HT) in different brain regions [58]. The present study supports the similar findings of our previous observations [50, 59]. CRS induced elevation of 5-HT levels were significantly attenuated by NJE (in both doses). The result also revealed that exposure to CRS significantly up regulated 5-HIAA levels in all three brain regions like cerebral cortex, hypothalamus and hippocampus. However, the increment of 5-HIAA level was found lower in hippocampus in comparison to other two brain regions. 5-HIAA level was measured higher in the hypothalamus and up regulated much higher than the cortex and hippocampus suggesting the fact that hypothalamus became more activated by the stress. A number of studies supported this finding that stressors augment serotonergic transmission in the median and dorsal raphe nuclei, brainstem, limbic regions such as hippocampus, hypothalamus, amygdala, frontal cortex etc. [60]. CRS induced alteration of 5-HIAA levels was significantly attenuated by NJE treatment. Further, result showed that in non-stressed animals, NJE treatment slightly increased the neurotransmitter levels including norpeinephrine, dopamine, serotonin and 5-Hydroxy indole acetic acid in all three brain regions, but not significantly. NJE treatment alone did not produce any significant change in the baseline values of these neurotransmitters; it restored the CRS mediated neurotransmitter alterations in the brain regions.

Earlier findings had shown that CRS acts through activation of H–P–A axis [15, 61]. Our previous study had shown that NJE provides protection against CRS through altering H–P–A pathway [4]. It has been reported that NE plays a role in the activation of H–P–A axis during stress [62]. The central serotonergic system influences hypothalamic corticotrophin releasing hormone (CRH) function and serotonergic neurotransmission is influenced by CRH [14, 63]. The catecholamine producing neurons innervates direct action upon CRH synthesizing neurons in the paraventricular neucleus (PVN) of hypothalamus during stress via noradrenergic bundle; which regulates the role of secretion of CRH/ACTH during stress [64]. Yoshida et al. (2010) has reported that restraint stress activates norepinephrine and serotonin neurons and also activates H–P–A axis [65]. Therefore, suggesting the fact that in this present result the activation of monoamines and the activations of H–P–A axis found in the previous study are interrelated, and support the above studies. It is reported that plasma corticosterone level, brain neurotransmitter concentration and oxidative changes are inter-connected, and regulates each other functions [66]. Therefore the down regulation of endogenous antioxidant system and up regulation of monoamines after CRS, suggesting the fact that, rapid metabolism of brain monoamines produces higher free radicals those are responsible for oxidative stress in the brain. Hence the present results are in conformity with earlier results.

Therefore, the above findings of the investigation indicated that NJE has the ability to overcome the stressful situations, and provides protection against CRS. Together with other indications of neuroprotective activities of NJE, this effect is providing evidence for its ant-stress property.

In this investigation we have administered *N. jatamansi* extract for 14 days to study the antistress effects against cold restraint stress. It has been well accepted that acute

stress is associated with improved arousal, which influences the animal for adaptive response by means of avoidance. We wanted to study whether the drug treatment enhances or blocks the adaptive phenomena after stress. In this study we have used subchronic drug treatment, because acute administration of drug may also produce adaptive response to the animals. Besides these, antipsychotic drugs (centrally acting drugs) generally show its efficacy after subchronic or chronic treatment. Therefore to study the effect on neurotransmitters, moreover to understand the neurotransmitters turn over (static and dynamic effect) subchronic exposure of drug treatment was adopted. In addition, we have used crude extract of jatamansi which is composed of several compounds, thereby offered pharmacological effects after repeated exposure. In our previous study we have used both 7 and 21 days treatment and found chronic treatment offered better effect [4, 26].

Conclusion

In conclusion, the above findings of the present investigation indicated that *N. jatamansi* has significant anti-stress activity against CRS. The modulatory effects of this drug have been investigated for the first time on the rat brain monoamines and oxidative processes after CRS. Mechanism of its action revealed that NJE executes the antistress effect through protection against oxidative stress. Therefore, antioxidant activity is one of modes by which it performs. The neurochemical alterations suggested the possible potential activities of the NJ on central nervous system. The pattern of the change of monoamines level after test drug treatment suggesting the fact that, the anti-stress activity attributed at least in part through altering the monoaminergic pathway.

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