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ORIGINAL ARTICLE

Neuroprotective efficacy of *Nardostachys jatamansi* and crocetin in conjunction with selenium in cognitive impairment

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Abstract Oxidative stress leads to complex biochemical alterations, and has been implicated in the progressive loss of learning and memory. Supplementing and boosting the endogenous antioxidant defense system could impede the progression of various types of neurodegeneration. In the present study, we have investigated the neuroprotective efficacy of a low-dose combination of certain promising and powerful natural antioxidants in an experimental model of cognitive impairment. Combined pretreatment with the extract of *Nardosatchys jatamansi* (N), crocetin (C) and selenium (Se) as sodium selenite (N, 200 mg/kg + C, 25 μg/kg + Se, 0.05 mg/kg body weight) for 15 days led to improved behavioral outcomes in streptozotocin (STZ)-induced cognitive impairment in rats. While intracerebroventricular (ICV) infusion of STZ resulted in the significant elevation of markers of oxidative stress and depletion of endogenous antioxidant defense system in the vehicle-pretreated group, these markers of oxidative stress and antioxidant enzymatic as well as non-enzymatic defense lines were attenuated in the group pretreated with the combination of antioxidants (NCSe). NCSe pretreatment markedly improved the performance of animals in passive avoidance test and Morris water maze (MWM) tasks, significantly reduced the level of TBARS, and elevated the content of glutathione and activities of antioxidant enzymes (glutathione peroxidase, glutathione-S-transferase and catalase). Our study reflects the synergistic potential of the above combination and concludes that a multimodal approach could be beneficial rather than a singular intervention.

Keywords Cognitive impairment · Crocetin · *Nardostachys jatamansi* · Neuroprotection · Oxidative stress · Selenium

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Introduction

Cellular stress and weakening of endogenous antioxidant defense are responsible for different neurodegenerative disorders, including Alzheimer's disease (AD) [1–3]. Disorders like AD are associated with a progressive loss of specific cholinergic neurons and the aggregation of beta amyloid and tau proteins.

Extensive oxidative stress leads to progression of different chronic disorders including cognitive impairment through neuronal dysfunction and death [4–6]. Therefore, retarding the oxidative stress could be a suitable strategy to

impede the progression of the diseases. The importance of antioxidant treatment in preventing neuronal damage has been emphasized somewhere [7–9].

Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats in sub-diabetogenic dose causes prolonged impairment of brain glucose and energy metabolism, and hence loss of neurons in the surrounding area, such as the hippocampus. This results in cognitive impairment due to decreased cholinergic activities induced by oxidative stress [10, 11].

The root and rhizomes of *Nardostachys jatamansi* (N) contain various sesquiterpenes, and their extract shows potent sedative, anti-stress and antioxidant effects on the central nervous system (CNS) [12–15]. A recent study has shown that jatamansi enhances learning and memory in mouse [16]. Our group has earlier reported the neuroprotective effects of jatamansi in the experimental models of Parkinsonism and cerebral ischemia–reperfusion [17, 18]. Various pharmacological effects of Jatamansi with special emphasis on the CNS have been reviewed elsewhere [19].

Saffron, *Crocus sativus* L., due to many different inherent pharmacological properties, has been used as, and in, folk medicine since ancient times [20]. Crocetin (C) and its by-product crocin (digentibiose adduct of crocetin) are the major active constituents of saffron extract. Their crucial pharmacological properties such as anticonvulsant, antinociceptive, anti-inflammatory and antidepressant properties have recently been reported [21–23]. It also shows free radical scavenging activities and enhances learning and memory [20, 24–27]. Saffron extract inhibits aggregation of beta amyloid in the brain [28]. Our group has reported preventive effect of crocetin at a dose as low as 25 μg/kg body weight in a rat model of hemi-parkinsonism [29].

Selenium (Se), a crucial trace element inside the body [30], has been reported to be neuroprotective at low dose in the different models of experimental neurodegeneration. Se, being a constituent of selenoproteins, is involved in antioxidant functions and helps to maintain redox homeostasis [31]. It is present at the site center of glutathione peroxidase (GPx) that protects the oxidative damage of membrane lipids and macromolecules due to peroxides [32]. It is also a major component for the catalytic activation of mammalian thioredoxin reductase [33]. Our group and others have reported the neuroprotective effects of low-dose Se, 0.1 mg/kg body weight, in experimental models of dementia [34] and 0.1–0.3 mg/kg body weight in other models of neurodegeneration [35, 36]. It has also been reported in positive clinical responses of Se therapy during neurodegenerative diseases [37, 38].

Recently, we have reported that Khamira Abresham Hakim Arshadwala, a complex approved recipe of Indian System of Medicine with many herbal and natural antioxidants present therein and multimodal effects, has encouraging results against STZ-induced cognitive impairment [39]. This prompted us to investigate a combination of certain promising antioxidants for the attenuation of learning and memory. The aim of the present study is to assess the neuroprotective effects of pretreatment with a low-dose combination of antioxidants from natural resources (Jatamansi extract, crocetin and selenium) in a rat model of cognitive impairment induced by ICV infusion of STZ.

Materials and methods

Plant extraction and preparation of drug

Roots of *Nardostachys jatamansi* were purchased from the herbal market of Delhi and were identified and authenticated by the taxonomist of the Department of Botany, Hamdard University, New Delhi. Clean roots were air dried, cut into small pieces and powdered to prepare the alcoholic extract as described earlier [40]. Briefly, 1 kg of powder was refluxed with 95% ethanol (1:10 w/v) for 8–10 h. The extract was evaporated to dryness using vacuum rotatory evaporator and stored at 4°C (yield: 10%). The dry extract was suspended in a mixture of ethanol: Tween 80:distilled water (1:2:5 v/v/v).

Animals

Male albino Wistar rats (450–500 g, aged 6–8 months) obtained from the Central Animal House Facility of Hamdard University were used as subjects. Rats were housed two per cage and kept in a temperature-controlled room with 12-h light and dark cycles. All rats were given free access to chow diet and water. All the experimental procedures performed on the animals were as per the guidance of the animal ethics committee of the university, a registered body with the Government of India with their authorized representative therein.

Chemicals

(−)-Epinephrine, glutathione (oxidized and reduced), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), 5-5′-dithiobis-2-nitrobenzoic acid (DTNB), sodium selenite, crocetin and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Chemical Private Ltd., India. All other chemicals were of analytical grade.

Experimental protocol and drugs

The animals were divided into four groups of eight rats in each. Treatment with the combination (NCSe) was given

between 10.00 a.m. and 12.00 noon every day by gavage for 15 days, followed by induction of cognitive impairment by ICV infusion of STZ on day 16. The first group, which was given only vehicle of the antioxidant combination orally up to 15 days, was sham (S). The group was ICV infused with 5 µl of normal saline (vehicle of STZ) to each of the lateral ventricles. The rats of the second group (L) were orally treated as above, but were ICV infused with STZ (3 mg/kg body weight in 5 µl saline) to each of the lateral ventricles. The third group (NCSe + L) was pretreated orally with a combination of NCSe (N = 200 mg/kg body weight, C = 25 µg/kg body weight and Na_2SeO_3 = 0.05 mg/kg) for 15 days, followed by ICV infusion of STZ as above on day 16. The fourth group (NCSe) was pretreated with NCSe orally for 15 days as above, but was infused only saline into each of the lateral ventricles as in the sham group. Passive avoidance test was started on day 14 post-ICV-STZ infusion until day 15. Morris water maze test was started from the noon of day 16 post-ICV-STZ infusion with a final trial on day 21. After finishing the test, animals were killed on the afternoon of the same day.

Induction of cognitive impairment

Cognitive impairment in Albino Wistar rats was induced as reported earlier [39, 41]. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg body weight in distilled water, IP) and placed in a stereotaxic frame. The head was positioned in a stereotaxic frame and a midline incision with sterile scalpel was made on the dorsal side of the skull. Burr holes were made in the skull on both the sides over the lateral ventricles using the coordinates: 0.9 mm posterior to the bregma; ± 1.5 mm lateral to the sagittal suture; 4.0 mm beneath the surface of the brain. STZ in a dose as mentioned above was slowly infused into both ventricles one at a time, into the relevant groups mentioned above. In the sham (S) and NCSe groups, STZ was replaced with physiological saline only.

Neurobehavioral experiments

Passive avoidance

Multiple-trial passive avoidance test was carried out on day 14 and 15 post-ICV-STZ infusion according to previous reports [42, 43] with slight modification. In brief, the apparatus consisted of an illuminated and a dark compartment. Both of them were equipped with a shock scrambler and grid floor, but separated by a guillotine door. In the acquisition trial, each rat was placed in the illuminated compartment. After 60 s of habituation, the guillotine door was opened and the initial latency (IL) to enter the dark chamber was recorded. Rats that had an initial latency time of more than 60 s were excluded from further experiment. When the animal entered the dark compartment, the door was closed and an electric foot shock (50 V, 0.2 mA, 50 HZ) was delivered to the floor grids for 3 s. The rat was removed from the dark chamber 5 s later and placed back into its home cage. The door was again opened 15 s later to start the next trial. After 24 h the retention latency (RL) time was measured in the same way as in the acquisition trial, but foot shock was not delivered, and the step-through latency time was recorded to a maximum of 600 s.

Morris water maze

Morris water maze test selected as an index for spatial learning and memory [44, 45] was started on the noon of day 16 post-ICV-STZ infusion. A circular tank (132 cm diameter, 60 cm height) was filled with water up to 40 cm and maintained at 27 ± 1°C. It was virtually divided into four equal quadrants as southwest (SW), southeast (SE), northeast (NE) and northwest (NW). The water was made opaque by the addition of white nontoxic water-soluble paints. A platform (10 cm × 5 cm) was placed in one of the quadrants and submerged 1–2 cm below the water surface. The water tank was surrounded by white curtain up to the top of the camera. Four light bulbs (100 W) were attached from the ceiling for sufficient lighting and were focused on the pool. A computerized digital tracking system (Columbus Instruments', Videomex-ONE, Ohio, USA) was used to record escape latencies and path length during each trial. Rats were randomly selected from one of the four groups. For each individual rat, the position of the platform was fixed during the entire experiment. The rats were trained with four trials per day for 6 consecutive days to locate and escape onto the platform. A different starting position for each rat was used in each trial. The rats were allowed to swim freely to find the hidden platform within 60 s and to stay on the platform for 30 s, and then returned to their cages until the next trial with a 10-min inter-trial interval. If a rat failed to locate the platform within 60 s, it was placed on the platform for the same interval of time as above during training. Escape latency times and distance traveled by the animals to reach the platform (path length) were recorded for each trial.

Probe trials on each rat were performed on the last day, 6th (day 21 post-ICV-STZ infusion), to assess the time spent in the target quadrant for memory retention after the learning trials. It was performed for a time period of 60 s without platform. The time spent in the quadrant (previously having platform in the training sessions) was taken as a measure of spatial memory.

Biochemical estimations

At the end of the behavioral studies in the afternoon of day 21 post-ICV-STZ infusion, the animals were killed. Brains were harvested to dissect hippocampi and frontal cortices quickly and stored at –80°C until estimations. Post-mitochondrial supernatant (PMS) prepared from 5% (w/v) homogenate (10,000*g* for 15 min at 4°C in 10 mM phosphate buffer, pH 7.0) was used in the analyses.

Estimation of thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation (LPO)

The method of Utley et al. [46] was used with minor modifications for the estimation of TBARS as an indicator of LPO by Islam et al. [47]. Briefly, 0.5 ml homogenate (5% w/v) was pipetted into a glass test tube (15 × 100 mm) and incubated at 37 ± 1°C in a metabolic water bath shaker for 60 min; another similar aliquot was placed at 0°C and marked as 0 h incubation. After 1 h of incubation, 0.5 ml of 5% TCA (chilled) and 0.5 ml of 0.67% thiobarbituric acid were added to each sample. The reaction mixture was centrifuged at 1,000*g* for 10 min. The supernatant was transferred to another tube and placed in a boiling water bath for 10 min. Thereafter, the tubes were cooled and the absorbance read at 535 nm. The rate of LPO was expressed as nmol of TBARS formed/h/g tissue using molar extinction coefficient of 1.5×10^5 M^{-1} cm^{-1}.

Estimation of reduced glutathione (GSH) level

Reduced glutathione content was determined by the method of Jollow et al. [48] with slight modifications. In brief, 0.5 ml of PMS was precipitated with 0.5 ml of 4% sulfosalicylic acid. The samples were kept at 4°C for 30 min. Thereafter, the mixture was centrifuged at 4,000*g* for 10 min and 0.1 ml of the supernatant was transferred to another test tube. To this, 0.2 ml of 5-5′-dithiobis-2-nitrobenzoic acid (4 mg/ml in 0.1 M phosphate buffer, pH 7.4) and 2.7 ml of 0.1 M phosphate buffer (pH 7.4) were added and vortexed. The yellow color developed was read immediately at 412 nm and the results expressed as nm of GSH/g tissue using a molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1}.

Estimation of glutathione peroxidase (GPx) activity

GPx activity was measured according to the procedure described by Mohandas et al. [49] The reaction mixture consisted of 1 mM of EDTA, 1 mM of sodium azide, 0.05 EU of GR, 1 mM glutathione, 0.2 mM of NADPH, 0.25 mM of hydrogen peroxide and 0.1 ml PMS (5% w/v) in a final volume of 2.0 ml with phosphate buffer (0.05 M, pH 7.0). The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated at nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Estimation of glutathione-S-transferase (GST) activity

GST activity was determined by the method of Habig et al. [50] as described by Athar et al. [51]. The reaction mixture consisted of 1 mM reduced glutathione, 1 mM CDNB and 0.30 ml PMS (5% w/v) in a total volume of 2.0 ml with phosphate buffer (0.1 M, pH 6.5). The changes in absorbance were recorded at 340 nm and the enzyme activity calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Estimation of superoxide dismutase (SOD) activity

SOD activity was measured as reported earlier by Steven et al. [52]. The auto-oxidation of (–) epinephrine at pH 10.4 for ~5 min was monitored at 480 nm against a blank. The reaction mixture contained 0.8 ml of 0.05 M glycine buffer (pH 10.4) and 0.2 ml PMS. The reaction was initiated by the addition of (–) epinephrine (0.02 ml of a 20 mg/ml solution). SOD activity was expressed as nmol of (–) epinephrine protected from oxidation by the sample. The molar extinction coefficient of 4.02 mmol l^{-1} cm^{-1} was used for calculations.

Estimation of catalase (CAT) activity

CAT activity was assayed by the method of Claiborne [53]. Briefly, the assay mixture contained 0.019 M hydrogen peroxide and 0.1 ml PMS in a total volume of 3.0 ml with 0.05 M phosphate buffer (pH 7.0). Change in absorbance was recorded at 240 nm. CAT activity was calculated as nmol H_2O_2 consumed/min/mg protein using a molar extinction coefficient of 43.6×10^3 M^{-1} cm^{-1}.

Estimation of protein

Protein concentrations were determined according to the method of Lowry et al. [54] using bovine serum albumin as standard.

Statistical analyses

Data are expressed as mean ± SEM. Statistical analysis was performed by using Origin Scientific Graphic and Analysis software, version 7.5. The physiologic data of each time point were analyzed by one-way ANOVA followed by Tukey–Kramer post hoc test for multiple

comparisons. The P values less than 0.05 were considered to be statistically significant.

Results

NCSe affects learning and memory in passive avoidance test in ICV-STZ induced rats

The results of passive avoidance test are summarized in Fig. 1. The mean initial latency on day 14 did not differ significantly between the sham (S), lesioned (L), NCSe + L and NCSe groups. On day 16, the mean retention latency in the lesioned group was significantly decreased ($P < 0.05$) as compared to the sham group. On the other hand, the NCSe + L group exhibited significant reversal ($P < 0.05$) of step-through latency as compared to the L group. The mean retention latency of the lesioned group was 218.75 ± 21.34 s, while in the NCSe + L group was 380 ± 20.51 s, showing significantly improved acquisition or retention of memory (Fig. 1) in the pretreated NCSe. No significant difference was observed between sham and NCSe groups.

Cognitively impaired animals pretreated with NCSe improved performance in MWM tasks

Figures 2 and 3, show the learning and memory restorative effects of the NCSe combination on escape latency and path length. A highly significant increase in escape latency (s) ($P < 0.001$) was observed in the L group, as compared to the S group, while pretreatment with NCSe in the NCSe + L group had significantly reduced escape latency ($P < 0.001$) as compared to the L group. No significant difference was observed in the NCSe group as compared to the S group (Fig. 2). A significantly ($P < 0.05$) longer path length (cm) was traveled by the L group as compared to the S group, while NCSe-pretreated NCSe + L group animals covered less than the group to reach the target platform ($P < 0.05$). Among the sham-operated groups, NCSe-pretreated group showed better response as compared to the S group, but was not significantly different (Fig. 3).

Data from the probe trials of the groups indicates the potential of NCSe efficacy for the retention of learning and hence memorizing the platform location (Fig. 4) in cognitively impaired animals. The L group rats spent very significantly ($P < 0.01$) less time in the target quadrant zone as compared to the S group. On the other hand, the rats pretreated with NCSe spent significantly ($P < 0.05$) more time in the target quadrant than the L group in the probe trial. No significant change in NCSe group rats was observed as compared to S group animals.

Biochemical studies

TBARS level in rat hippocampus and frontal cortex

The content of TBARS in hippocampus and frontal cortex was significantly ($P < 0.05$) elevated in L group as

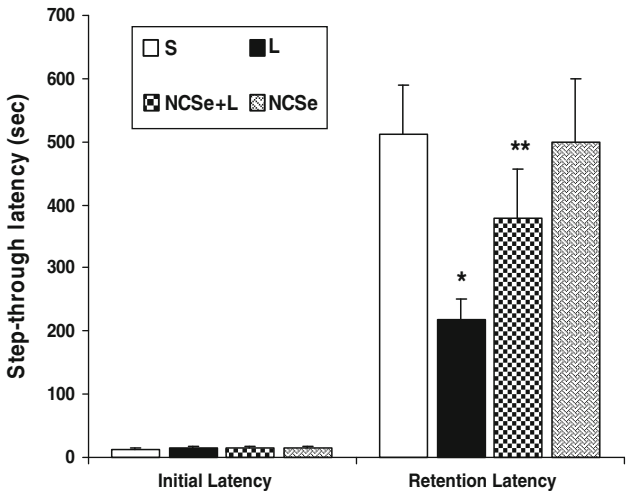

Fig. 1 Effect of pretreatment of NCSe ($N = 200$ mg/kg body weight, $C = 25$ µg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg) on passive avoidance paradigm in rats. Values are expressed as mean ± SE. *$P < 0.05$, retention latency of L group rats versus that of S group; **$P < 0.05$, L group versus NCSe + L group

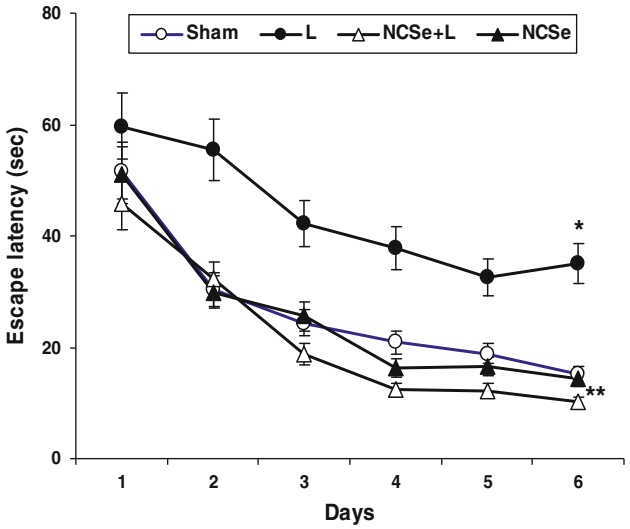

Fig. 2 Effect of ICV-STZ injection on learning and memory disability in the L group rats and their prevention by the pretreatment with NCSe ($N = 200$ mg/kg body weight, $C = 25$ µg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg). Values are expressed as mean ± SE ($n = 8$) of the time required to find an invisible platform submerged in Morris water maze task of four trials per day (escape latency). *$P < 0.001$ S versus L, **$P < 0.001$ L versus NCSe + L

compared to S group, and reduced significantly ($P < 0.05$) in NCSe-pretreated cognitively impaired group as compared to L group. There was insignificant difference between sham-operated two groups (Fig. 5).

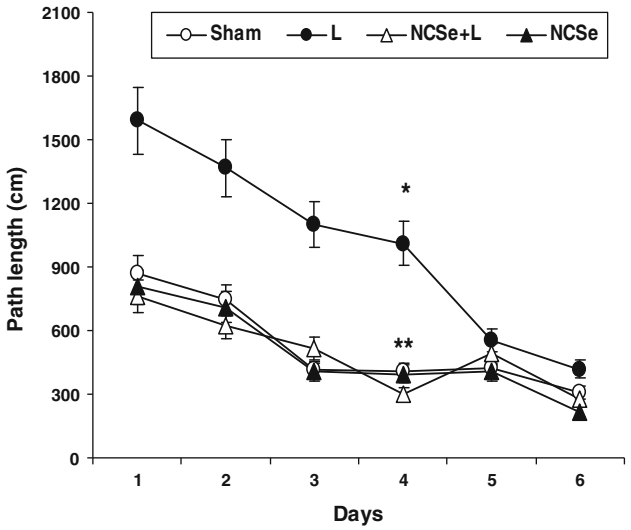

Fig. 3 Effect of pretreatment of NCSe ($N = 200$ mg/kg body weight, $C = 25$ μg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg) on acquisition learning and memory in ICV-STZ treated L group (NCSe + L). The results are expressed as mean ± SE of the path length to find the platform in the Morris water maze task of four trials on each day ($n = 8$ rats/group). *$P < 0.05$ S versus L; **$P < 0.05$, L versus NCSe + L

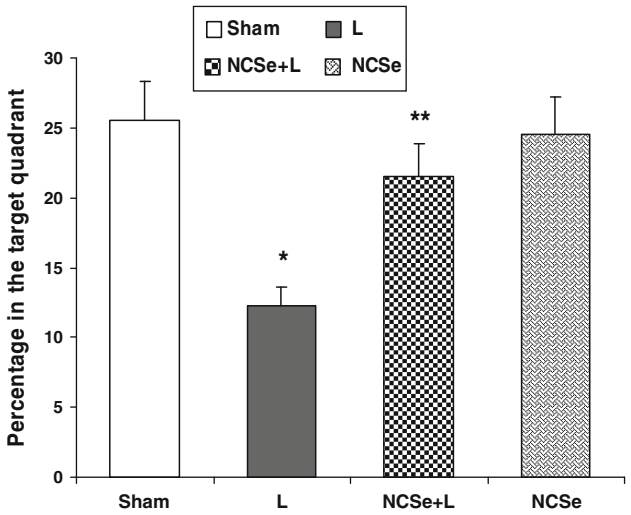

Fig. 4 Effect on learning and memory (cognitive impairment) after pretreatment with NCSe ($N = 200$ mg/kg body weight, $C = 25$ μg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg) in ICV-STZ treated L group (NCSe + L) on the mean percentage time spent in the target quadrant in which the earlier platform was taken during trial in Morris water maze navigation task. NCSe has significantly protected the ICV-STZ induced memory deficits in the NCSe + L group rats. Data are expressed as mean ± SE ($n = 8$). *$P < 0.01$ S versus L group; **$P < 0.05$ L versus NCSe + L group

Glutathione level in rat hippocampus and frontal cortex

The GSH levels were found significantly decreased ($P < 0.01$) in L group as compared to S group, while it was significantly elevated ($P < 0.01$) in NCSe + L group as compared to L group (Fig. 6).

Activities of antioxidant enzymes

The mean activities of GPx, GST, SOD and CAT were very significantly ($P < 0.01$) depleted in hippocampus (Table 1) and frontal cortex (Table 2) in the L group as compared to the S group, while their activities were significantly ($P < 0.05$) attenuated in the NCSe + L group as compared to the L group. No significant changes were observed in the sham-operated NCSe group as compared to the S group.

Discussion

Free radical induced oxidative stress may results in progressive decline in cognition [55, 56] which could be slowed down through antioxidant supplement. Here, we report the synergistic effects of low dose of *Nardostachys jatamansi* extract, crocetin and selenium (NCSe) in combination in a rat model of experimental dementia. NCSe when

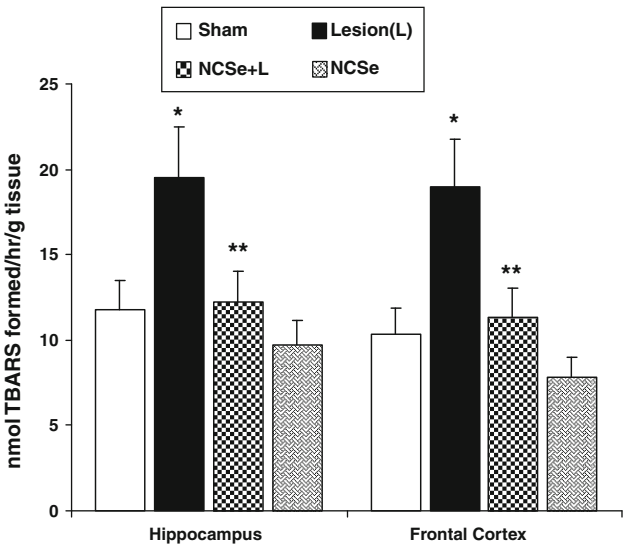

Fig. 5 Effect of pretreatment of NCSe ($N = 200$ mg/kg body weight, $C = 25$ μg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg) on the content of LPO in the hippocampus and frontal cortex in ICV-STZ treated L group (NCSe + L). Data are expressed as mean ± SE ($n = 8$). ICV-STZ significantly increased the TBARS in the L group as compared to the S group rats. Pretreatment of NCSe significantly reduced TBARS content in the NCSe + L group as compared to the L group. Unit of LPO is expressed as nmoles of TBARS/h/g tissue. *$P < 0.05$ S versus L group; **$P < 0.05$ L versus NCSe + L group

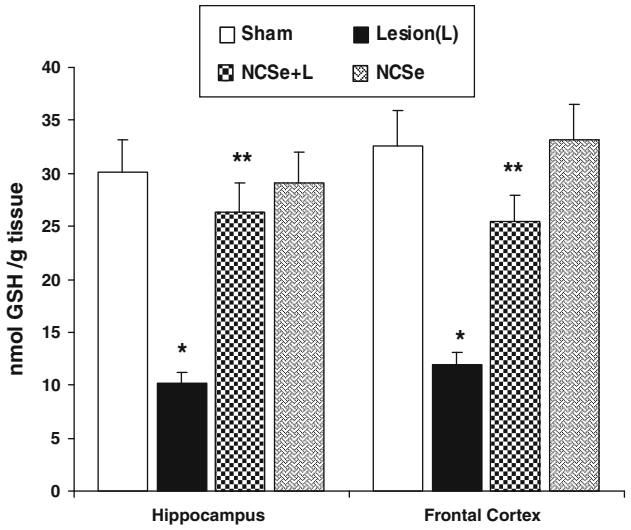

Fig. 6 Effect of pretreatment of NCSe ($N = 200$ mg/kg body weight, $C = 25$ μg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg) on the content of GSH in the hippocampus and frontal cortex in ICV-STZ treated group (NCSe + L). Values are expressed as mean ± SE ($n = 8$). Unit of GSH is expressed as μg GSH/g tissue. *$P < 0.01$ sham versus L, **$P < 0.01$ L versus NCSe + L

pretreated orally in aged rats prevented ICV-STZ induced loss of cognition through attenuation of oxidative stress.

ICV-STZ infusion in sub-diabetogenic dose leads to failure of cellular energetics due to oxidative stress, and has been used as a model of sporadic dementia [57, 58]. It decreases the local glucose, ATP level, AChE and ChAT activity, [34, 59, 60] and also alters calcium influx [61, 62].

Pretreatment of ICV–STZ infused old rats with NCSe reversed the impaired learning and memory in two separate behavioral paradigms. In passive avoidance (Fig. 1) and MWM navigation tasks (Figs. 2, 4), NCSe pretreatment preserved learning and memory significantly as compared to the vehicle-pretreated group. In the MWM task, the ICV-STZ infused NCSe-pretreated animals showed significant difference both in escape latency (Fig. 2) and path length (Fig. 3) as compared to the vehicle-pretreated ICV-STZ group. In the probe test of MWM, ICV-STZ infused animals without NCSe pretreatment spent less time in the target quadrant due to loss of memory than the sham group (Fig. 4), while the loss was preserved in NCSe-pretreated group. Collectively, both the tests reveal that the cognitive performance is restored in the NCSe-pretreated group.

ICV-STZ infusion leads to highly reactive ROS production increasing lipid peroxidation, leading to cellular disintegrity and progressive dementia. Lipid peroxides and hydroperoxides cause secondary injury by further generating relatively more stable and diffusible cytotoxic agents, such as malondialdehyde (MDA) and 4-hydroxy-*trans*-2-nonenal (4-HNE), respectively, and amplify the oxidative cascade. TBARS and F2-isoprostanes have been reportedly increased in AD brains [63]. They react avidly with cellular nucleophiles such as glutathione (GSH) and cause continuous decrease in their level through increased oxidant content or protein modification. Figures 5 and 6 show that ICV-STZ infusion very significantly elevated the level of TBARS and decreased the glutathione content. Pretreatment with NCSe significantly decreased the TBARS content and elevated glutathione level.

Depleted cellular enzymatic activities are also used as markers of oxidative stress. Consistent with the previous reports in the hippocampus, [39, 41] and we observed highly decreased activities of these antioxidant enzymes (GPx, GR, GST, SOD and CAT) in ICV-STZ infused vehicle rats. Pretreatment of the animals with NCSe combination ameliorated the sustained oxidative stress through up-regulation of the activities of these enzymes (Tables 1, 2).

Jatamansi extract has been reported (200–600 mg/kg body weight, orally) to improve learning and memory in mice [16] and ameliorate other neurodegenerative cascades in rats [17, 18]. We have also reported the neuroprotective effects of crocetin (25, 50 and 75 μg/kg body weight) [29] and selenium (0.05, 0.1, 0.2 and 0.3 mg/kg body weight)

Table 1 Hippocampus: effect of cognitive impairment on the activity of antioxidant enzymes and their protection with NCSe ($N = 200$ mg/kg body weight, $C = 25$ μg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg)

Parameters	Sham (S)	Lesion (L)	NCSe + L	NCSe only
GPx (nmol NADPH oxidized/min/mg protein)	676.12 ± 88.06	347.01 ± 25.45* (−48.67%)	569.15 ± 43.05# (+64.01%)	669.01 ± 47.44 (−1.06%)
GST (nmol CDNB conjugate formed/min/mg protein)	667.13 ± 47.48	405.39 ± 26.3* (–39.23%)	588.91 ± 64.95# (+45.26%)	646.29 ± 20.58 (–3.12%)
SOD (nmol epinephrine protected from oxidized/min/mg protein)	782.01 ± 40.14	495.39 ± 20.3* (–36.56%)	758.91 ± 60.95# (+53.19%)	786.29 ± 20.58 (+0.5%)
CAT (μmole H_2O_2/min/mg protein)	50.12 ± 9.21	10.23 ± 1.3* (–79.58%)	44.57 ± 4.2# (+335.67%)	51.12 ± 7.5 (+1.9%)

Values are expressed as mean ± SE. Values in parentheses show the percentage increase or decrease with respect to their control

* $P < 0.01$ S versus L, # $P < 0.05$ L versus NCSe + L

Table 2 Frontal cortex: effect of cognitive impairment on the activity of antioxidant enzymes, and their protection with NCSe ($N = 200$ mg/kg body weight, $C = 25$ μg/kg body weight and $Na_2SeO_3 = 0.05$ mg/kg)

Parameters	Sham (S)	Lesion (L)	NCSe + L	NCSe only
GPx (nmol NADPH oxidized/min/mg protein)	555.56 ± 72.35	307.11 ± 22.52* (–44.72)	498.1 ± 37.67# (+62.18%)	575.22 ± 40.78 (+3.53%)
GST (nmol CDNB conjugate formed/min/mg protein)	656.12 ± 40.21	398.56 ± 23.4* (–39.25%)	506.23 ± 60.23# (+27.01%)	625.36 ± 19.35 (+4.68%)
SOD (nmol epinephrine protected from oxidized/min/mg protein)	726.12 ± 38.21	498.56 ± 19.4* (–31.33%)	646.23 ± 52.23# (+29.61%)	735.36 ± 15.35 (+1.27%)
CAT (μmole H_2O_2/min/mg protein)	48.33 ± 8.21	7.98 ± 1.2* (–83.48%)	43.44 ± 5.3# (+444.36%)	49.13 ± 5.35 (+1.65%)

Values are expressed as mean ± SE. Values in parentheses show the percentage increase or decrease with respect to their control

* $P < 0.01$ S versus L, # $P < 0.05$ L versus NCSe + L

in acute and chronic models of neurodegeneration [34, 35, 64, 65]. One of the common effects of these nutraceuticals is the attenuation of increased oxidative stress. While jatamansi was used in CNS disorders and promoted neurotrophic activity [66], selenium was found to ameliorate decreased ChAT activity in the hippocampus [34] after ICV-STZ infusion in rats, and crocetin was a potent antioxidant. Selenium has been detected at the site center of GPx and other selenoproteins, which play a significant protective role against oxidative stress. Traditional medicines are mostly crude in preparation and might have undesirable side effects at higher doses, while low dose may compromise the efficacy. The combination of many constituents may provide better protection against neurodegeneration [36, 39]. Here, we anticipate that this combination will divert most of jatamansi's restorative effects to the brain for memory preservation, while crocetin and selenium will act as supplements to reduce oxidative stress.

In conclusion, the improved behavioral outcomes after NCSe pretreatment in animals encourage the use of polytherapy to prevent cognitive impairment in aged individuals. However, further research is required to determine their optimum efficacy and mechanism of action to achieve the desired, but safe, pharmacological effects.

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