Nardostachy Jatamansi and Crocetin in Conjunction with Selenium has Potent Neuroprotection in Cognitive impairments (Alzheimer’s Disease)
INTRODUCTION

Neurodegenerative diseases, such as Alzheimer's disease (AD) are associated with a progressive loss of specific neuronal cells population coupled with protein aggregates. AD is characterized by extensive oxidative stress, which might be responsible for the dysfunction or death of neuronal cells that contribute to disease pathogenesis. Oxidative stress has been demonstrated to be related to the pathophysiological consequences of brain injury in common neurodegenerative disorders, including AD (Mates et al., 1999; Alexi et al., 2000; Halliwell and Gutteridge, 1999).

Intracerebroventricular (ICV) injection of streptozotocin (STZ) in sub diabetic dose in rats has been found to cause prolonged impairment of brain glucose and energy metabolism. This is accompanied by impairment in learning and memory in addition to decreased choline acetyltransferase activity in the hippocampus (Blokland and Jolles, 1993; Lannert and Hoyer, 1998).

Aggregating evidence suggests that cellular stress induced by free radicals is responsible for a variety of neurodegenerative disorders of central nervous system (CNS), including AD and in pathological conditions such as cerebral ischemia and excitotoxicity (Halloween, 1992; Coyle and Puttfarcken, 1993; Olanow, 1993). The importance of treatment with antioxidant in preventing oxidative stress induced neuronal damage has recently been emphasized (Ureno et al., 1997; Yamamoto et al., 1997; Bastianetto et al., 1999).

Nardostachys jatamansi (Valerianaceae), popularly known as "Jatamansi", is native to the Alpine Himalayas. The rhizomes and roots are used in the Indian System of Medicine as a sedative and in anistress remedy (Chatterjee and Prakashi, 1997). Various sesquiterpenes (such as Jatamansic acid and Jatamansone), lignans, and neolignans have been reported to be present in the roots of the plant (Chatterji and Prakash, 1997; Arora, 1965; Rastogi and Mehrotra, 1990) with distinct depressant action on the CNS and antioxidant effects (Tripathi et al., 1996). Recent study shows that Nardostachys jatamansi (N) enhanced learning and memory in exteroceptive as well as interoceptive models of mice (Joshi and Parle, 2006).

From this lab Salim et al., (2003) have reported the protective role of N in cerebral ischemia by scavenging the free radical activities. Crocetin (C) is an active principle of Crocus sativus L (saffron), and used in folk medicine as an antispasmodic, eupeptic, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac and emmenagogue (Rios et al., 1996). Moreover, modern pharmacological investigations have reported that saffron extract or its active constituents have anticonvulsant (Hosseinzadeh and Khosravan, 2002), antidepressant (Hosseinzadeh et al., 2004), anti-inflammatory (Hosseinzadeh and Younesi, 2002) and free radical scavenging activities as well as learning and memory improving properties (Rios et al., 1996; Abdullaei, 1993; Escribano et al., 1996; Zhang et al., 1994; Abe et al., 1999). Because of its powerful antioxidant activity, its active principle, crocetin could be useful in the therapy of brain neurodegenerative disorders, as its effects have been evaluated in hemi-Parkinson’s disease (Ahmad et al., 2005).

In order to maintain over all body defense mechanism of immune system, it is apparent that selenium (Se), as an essential trace element (Schwarz and Folz, 1957), also plays a significant role in the protection of nervous system at low doses. Se is a constituent of selenoprotein which is involved in antioxidant function and redox status (Brauer and savaskan, 2004). On the other hand, Se is present at the site center of glutathione peroxidase (GPxs), an antioxidant enzyme that protects membrane lipids and macromolecules from oxidative damage produced by peroxides, such as hydrogen peroxide (Harman, 1993). Se is also a major component of a thioredoxin reductase, an important antioxidant protein for mammals (Lee et al., 2000). Neuroprotective effects of Se have been reported in 6-hydroxydopamine...
Parkinsonian models of rats (Zafar et al., 2003) as well as in positive clinical responses of Se therapy during neurodegenerative diseases (Halliwell and Gutteridge, 1984; Westermarck and Santavouri, 1984).

The aim of the present study is to assess the potent protective role of combined multiple antioxidants in low doses against Streptozotocin - induced cognitive impairment in rat models of neurodegeneration.

MATERIAL AND METHODS

Plant extraction and preparation of drug

Roots of *Nardostachys jatamansi* (N) were purchased from the herbal market of Delhi and were identified and authenticated by the expert taxonomist of the Department of Botany, Jamia Hamdard (Hamdard University), New Delhi. Clean roots were air-dried cut in to small pieces and powdered to prepare the alcoholic extract as described earlier (Salim et al., 2003). Briefly, one kilogram of powdered roots of *jatamansi* 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 consisting of ethanol: Tween 80: distilled water (1:2:5 v/v/v).

Drugs and 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 (Se), crocin and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Chemical private Ltd., India. All other chemicals were of analytical grade.

Animals

Male albino Wistar rats (450-500 g, aged 6-8 months) obtained from Central Animal House, Jamia Hamdard (Hamdard University), New Delhi were used as subjects. Rats were housed two per cage and kept in a temperature-controlled room with light-dark cycle (12 hr). All rats were given free access to water ad libitum.

Experimental protocol

The animals were divided into four groups of eight rats each. The first group was sham (S), and normal saline 5μl was injected ICV to each lateral ventricle. The rats of the second group received a single injection of ICV-STZ (3 mg/kg bwt/5μl) to each lateral ventricle. The third group was pretreated orally with a combination of N (200mg /kg b wt), C (25 μg/kg b wt) and Se (0.05 mg/kg b wt) for 15 days. The fourth group was pretreated with combined drugs NCSe orally. Treatment of NCSe was given between 10.00 and 12.00 hr every day using an intragastric cannula for 15 days. On day 16th animals were injected ICV-STZ.

Induction of Cognitive Impairments (Alzheimer’s disease)

![Figure 2.1 Animal on dual manipulator stereotaxic instrument attached with 10 μl Hamilton syringe and fixed drill machine during surgical procedure for the induction of cognitive impairments.](image-url)
lateral ventricles using the following coordinates: 0.9 mm posterior to bregma; ±1.5 mm lateral to sagittal suture; 4.0 mm beneath the surface of the brain. Streptozotocin (3 mg/kg body weight/) was injected ICV bilaterally at 5μl injection/site to the lesioned group (L) and combined drugs + lesioned group (NCSe+L). In the sham and NCSe group, physiological saline (0.9%) was injected (5 μl on each site) in the same way as in the L group.

Behavioral Analysis

Effect of NCSe on learning and memory

Passive avoidance

Multiple-trial passive avoidance test was carried out on days 14-15 after the start of ICV-STZ infusion according to previous methods (Olariu et al., 2001; Sharma and Gupta, 2003) with slightly modification. In brief, the apparatus consisted of two compartments, one illuminated and other dark, both equipped with a shock scrambler, grid floor. A guillotine door separated the two compartments. In the acquisition trial, each rat was placed in the illuminated compartment. After 60 s of habituation, a guillotine door separating the lighted and dark chambers 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. 24 h later the retention latency (RL) time was measured in the same way as in the acquisition trial, but foot shock was not delivered, and the latency time was recorded to a maximum of 600 s.

Morris water maze

Two weeks after the surgery, the Morris water maze (Morris, 1984; Gallagher, 1993) was selected as an index for spatial learning and memory. A circular tank (132 cm diameter, 60 cm height) was filled to a depth of 40 cm with water at 27±1°C, and the tank was divided virtually into four equal quadrants such as south-west (SW), south-east (SE), north-east (NE) and north-west (NW). The water was made opaque by the addition of white nontoxic water-soluble paints. A platform (10 cm x 5 cm) was placed in one of the four maze quadrants and submerged 1-2 cm below the water surface.

The water tank from the pool-wall was surrounded with 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 directly on the pool. A computerized digital tracking system (Columbus Instruments, Videome-ONE, Ohio, USA) was used to record escapes 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 for 4 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 after reaching the platform they were allowed to stay on the platform for 30 s, and then return to the cage, and to await their next trial at 10-minutes interval. If a rat failed to locate the platform within 60 s it was then placed on it for the same interval of time. Latency times and distance traveled by the animals to reach the platform (path length) were recorded for each trial.

Probe trial

The purpose of probe trail was to assess the time spent in the target quadrant for memory retention after learning. One day after the last training trial, animals were subjected to probe trial for 60 s without platform. The time spent in the quadrant of the former platform position was taken as a measure for spatial memory.
Tissue preparation

After behavioral study, the animals were sacrificed on day 21 and their brains were taken out to dissect hippocampus and frontal cortex quickly and stored at -80 °C until further use. Post mitochondrial supernatant (PMS) obtained from 5% (w/v) homogenate (10,000 g for 15 min at 4 °C in 10 mM phosphate buffer, pH 7.0) was used for the estimation of various parameters related to oxidative stress.

1. Non-enzymatic Assays

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

The method of Utely et al. (1967) was used for the estimation of lipid peroxidation as modified in our laboratory (Islam et al., 2002). Briefly 0.5 ml homogenate (5% w/v) was pipetted in a glass test-tube (15×100 mm) and incubated at 37±1°C in a metabolic water bath shaker for 60 min, another aliquot of 0.5 ml was pipetted in a centrifuge tube and placed at 0 °C and marked as 0 hours incubation. After 1 hour of incubation, 0.5 ml of 5% TCA (chilled) and 0.5 ml of 0.67% thiobarbituric acid was added in each sample (i.e., 0 °C and 37 °C). The reaction mixture was centrifuged at 1000 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 lipid peroxidation was expressed as nmol TBARS formed/h/g tissue using molar extinction coefficient of 1.5 x 10^5 M^-1 cm^-1.

Estimation of reduced glutathione (GSH)

Reduced glutathione in the hippocampus and frontal cortex was determined by the method of Jollow et al. (1974) 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 4000 g for 10 min and 0.1 ml 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) was 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 x 10^3 M^-1 cm^-1.

2. Enzymatic Assays

Estimation of glutathione peroxidase activity

GPx activity was measured according to the procedure described by Mohandas et al. (1984). The reaction mixture consisted of 1 mM of EDTA, 1 mM of sodium azide, 1.4 U of GR, 1 mM of 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 x 10^3 M^-1 cm^-1.

Estimation of glutathione-S-transferase (GST) activity

Glutathione-S-transferase activity was determined by method of Habig et al. (1974) as described by Athar et al. (1989). 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 was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 x 10^3 M^-1 cm^-1.

Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured by the method of Steven et al. (2000). The auto-oxidation of (-)-epinephrine for ~5 min at pH 10.4 was monitored at 480 nm. The reaction mixture contained 0.8 ml of
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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 compared with the corresponding readings of the blank. The molar extinction coefficient of 4.02 mmol·l⁻¹·cm⁻¹ was used for the calculations.

Estimation of catalase (CAT) activity

CAT activity was assayed by the method of (Claiborne, 1985). 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 in terms of nmol H₂O₂ consumed/min/mg protein using a molar extinction coefficient of 43.6 x 10⁴ M⁻¹·cm⁻¹.

Estimation of protein

Protein concentrations were determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Data are expressed as using Origin Scientific Graphic and Analysis software, version 7.5, performed mean ± SE statistical analysis. 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 statistically significant.

RESULTS

Effect of NCSe on learning and memory

NCSe protects the multiple-trial passive avoidance test in ICV-STZ induced rats

Learning and memory was tested in the multiple-trial passive avoidance paradigm on day 14 to 15 after the start of ICV-STZ infusion. The mean initial latency on day 14 did not differ significantly in sham (S), Lesioned (L), NCSe+L and NCSe groups. The initial latency was 13.5 ± 0.64, 16 ± 0.70, 15.5 ± 1.04, 14.5 ± 0.64 s, respectively.

On day 15, the mean retention latency in ICV-STZ infused rats, i.e., lesioned group was significantly decreased (P<0.05) as compared to the sham group, the retention latency being 218.75 ± 21.34 and 512.5 ± 16.13 s, respectively. On the other hand, the group L that was treated with NCSe (NCSe+L) exhibited significant reversal (P<0.05) of transfer latency as compared to L group. The mean retention latency of NCSe+L group was 380 ± 20.51. However, the effect was significant on NCSe administration, which was significantly higher than ICV-STZ infusion 1. group indicating improved acquisition or retention of memory (Fig. 2.2). No significant effect was found in the steps—through latencies or number of trials among NCSe group as compared to sham group.

Figure 2.2 Effect of pretreatment of NCSe on passive avoidance paradigm in rats. Values are expressed as mean ± S.E. *P<0.05, retention latency of L group rats vs S group; #P<0.05, L group vs NCSe+L group.

Morris water maze

Escape latency

Figure 2.3 and 2.4 shows the potential effect of the combined drugs NCSe on escape latency and path length in ICV-STZ induced neurodegeneration (learning and memory deficit) in the S and L groups and restoration afforded by NCSe.
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A significant increase in escape latency (s) ($P<0.001$) was observed in L group, as compared to S group, while treatment with NCSe in L group, i.e., (NCSe+L) group have significantly reduced escape latency as compared to L group. No significant difference was observed in NCSe group as compared to S group (Figure 2.3).

Figure 2.3 Effect of ICV-STZ injection on learning and memory disability in L group rats and their prevention by the pretreatment with NCSe. Values are expressed as mean ± S.E. (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 vs L; #$p<0.001$ I. vs NCSe+L.

Path length

A significantly ($P<0.05$) long path length (cm) was covered by L group as compared to S group while NCSe treated L group, i.e., (NCSe+L) exhibited a significant reduction in path length. The NCSe group showed better response as compared to S group. (Figure 2.4)

Probe trial

Data from the probe trial of the Morris water maze navigation task indicated a significantly enhanced performance in learning and platform location during the six days of training of the animals of different groups (Fig.2.5). The L group rats spent 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 trail.

Figure 2.4 Effect of pretreatment of NCSe on acquisition learning & memory in ICV-STZ treated L group (NCSe+L). The results are expressed as mean± S.E. of the path length to find the platform in Morris water maze task of four trials on each day (n=8 rats/group) *$p<0.05$ S vs L; #$p<0.03$ L. vs NCSe+L.

No significant change in NCSe group rats was observed as compared to S group rat.

Figure 2.5 Effect of learning and memory (cognitive impairment) after pretreatment with NCSe 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 NCSe+L group rats. Data are expressed as mean± S.E. (n=8). *$p<0.01$ S vs L group; #$p<0.05$ I. vs NCSe+L group

BIOCHEMICAL PARAMETERS

TBARS level in rat’s hippocampus and frontal cortex

The content of TBARS in the hippocampus and frontal cortex was significantly ($P<0.05$)
Glutathione level in rat's hippocampus and frontal cortex

The protection of NCSe on GSH level was demonstrated in the hippocampus and frontal cortex. The GSH levels were significantly decreased ($P<0.01$) in L group as compared to S group, where the corresponding levels in NCSe+L. group was significantly elevated ($P<0.01$) as compared to L group. There was no significant change in GSH content of NCSe group as compared to S group (Figure 2.7).

Activity of antioxidant enzymes in rat's hippocampus and frontal cortex

The activity of GPx, GST, SOD and CAT was significantly ($P<0.01$) depicted in hippocampus (Table 2.1) and frontal cortex (Table 2.2) in L group rats as compared to S group, where as its activity was significantly ($p<0.05$) increased in NCSe+L group as compared to L group. No significant change was shown in NCSe group as compared to S group.
### Table 2.1 Hippocampus

Effect of cognitive impairment on the activity of antioxidant enzymes, and their protection with NCSe

<table>
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<th>Parameters</th>
<th>Sham (S)</th>
<th>Lesion (L)</th>
<th>NCSe +L</th>
<th>NCSe only</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (nmol NADPH oxidized / min / mg protein)</td>
<td>676.12 ± 88.06</td>
<td>347.01 ± 25.45* (−48.67%)</td>
<td>569.15 ± 43.05# (+64.01%)</td>
<td>669.01 ± 47.44 (−1.06%)</td>
</tr>
<tr>
<td>GST (nmol CDNB conjugate formed / min / mg protein)</td>
<td>667.13 ± 47.48</td>
<td>405.39 ± 26.3* (−39.23%)</td>
<td>588.91 ± 64.95# (+45.26%)</td>
<td>646.29 ± 20.58 (−3.12%)</td>
</tr>
<tr>
<td>SOD (nmol epinephrine protected from oxidized / min / mg protein)</td>
<td>782.01 ± 40.14</td>
<td>495.39 ± 20.3* (−36.56%)</td>
<td>758.91 ± 60.95# (+53.19%)</td>
<td>786.29 ± 20.58 (+0.5%)</td>
</tr>
<tr>
<td>CAT (μmole H₂O₂ consumed / min / mg protein)</td>
<td>50.12 ± 9.21</td>
<td>10.23 ± 1.3* (−79.58%)</td>
<td>44.57 ± 4.2# (+335.67%)</td>
<td>51.12 ± 7.5 (+1.9%)</td>
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</tbody>
</table>

Values are expressed as mean ± S.E. Values in the parentheses show the percentage increase or decrease with respect to their control. *p < 0.01 S vs. L; #p < 0.05 L vs. NCSe + L

### Table 2.2 Frontal Cortex

Effect of cognitive impairment on the activity of antioxidant enzymes, and their protection with NCSe

<table>
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<tr>
<th>Parameters</th>
<th>Sham</th>
<th>Lesion (L)</th>
<th>NCSe +L</th>
<th>NCSe only</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (nmol NADPH oxidized / min / mg protein)</td>
<td>555.56 ± 72.35</td>
<td>307.11 ± 22.52* (−44.72)</td>
<td>498.1 ± 37.67# (+62.18%)</td>
<td>575.22 ± 40.78 (+3.53%)</td>
</tr>
<tr>
<td>GST (nmol CDNB conjugate formed / min / mg protein)</td>
<td>656.12 ± 40.21</td>
<td>398.56 ± 23.4* (−39.25%)</td>
<td>506.23 ± 60.23# (+27.01%)</td>
<td>625.36 ± 19.35 (+4.68%)</td>
</tr>
<tr>
<td>SOD (nmol epinephrine protected from oxidized / min / mg protein)</td>
<td>726.12 ± 38.21</td>
<td>498.56 ± 19.4* (−31.33%)</td>
<td>646.23 ± 52.23# (+29.61%)</td>
<td>735.36 ± 15.35 (+1.27%)</td>
</tr>
<tr>
<td>CAT (μmole H₂O₂ consumed / min / mg protein)</td>
<td>48.33 ± 8.21</td>
<td>7.98 ± 1.2* (−83.48%)</td>
<td>43.44 ± 5.3# (+444.36%)</td>
<td>49.13 ± 5.35 (+1.65%)</td>
</tr>
</tbody>
</table>

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DISCUSSION

Free radicals-induced oxidative stress and damage are the important factors for both, aging and the cognitive impairments of neurodegenerative disorders (Zs-Nagy, 1978; Zs-Nagy, 1990). In the present study, we investigated the synergistic effect of potent drugs Nordostacbj Jatamansi (N), Crocetin (C) and selenium (Se) [NCSe] in the prevention of cognitive impairments and oxidative damage caused by ICV-STZ in rats. STZ treated rats were used as animal models of sporadic AD (Blokland et al., 1993; Duelli et al., 1994; Nitsch and Hoyer, 1991; Plaschke and Hoyer, 1993).

The pretreatment of ICV–STZ treated old rats with combined drugs, NCSe at concentrations lower than that of drugs alone could reverse the impaired learning and memory in two separate behavioral paradigms. In the foot shock passive avoidance task as well as Morris water maze navigation task, pretreatment with NCSe was found to return learning to a level that was not different from sham. In the Morris water maze task, the ICV-STZ induced animals showed significant difference both in escape latency and path length as compared to the other three groups. Moreover, in the probe test of Morris water maze task, the animals treated with ICV-STZ spent less-time in the platform quadrant than the sham group rats, although there was no significant difference in performance during the course of the initial training trials among the three groups. The memory retardation was not due to an impairment of swimming ability, since in the first trial of the initial learning tests, the pattern and the latency to search the hidden platform did not differ between ICV-STZ treated compared with the other three groups (S, NCSe+L and NCSe). However, the initial trial of Morris water maze spatial test and subsequent trail to reach the platform was significantly different in L and sham group. Collectively, it may be suggested that streptozotocin disrupts spatial memory. The results of the water maze test were confirmed by the multiple passive avoidance tasks. The ICV-STZ induced a significant reduction of the step-through latency in the retention test, suggesting a deficiency in the learning and memory to remember the experience of the electric foot shock.

The potential of antioxidants to reverse cognitive impairments depends upon their ability to cross the blood brain barrier (BBB) and reach the brain. The aggregation of free fatty acids (FFA) in the cortical and hippocampal areas of the brain with intracerebroventricularly administered STZ (Muller et al., 1998) and from a complex interplay between free and serum protein bound FFA/BBB–influx and BBB efflux transporters, and brain utilization (Banks et al., 1997; Rapoport and Robinson, 1986).

We assessed the impact of antioxidants’ treatment on the oxidative stress biomarkers in the L group rats with neurodegeneration induced by intracerebroventricularly administered STZ. The ICV-STZ infused rats had altered all the oxidative stress biomarkers as compared to sham. Further, ICV-STZ group pretreated with combined NCSe, antioxidants has significantly balanced all the oxidative stress biomarkers as compared to ICV-STZ group. The results reflect that oxidative stress is widespread, affecting measures of lipid peroxidation, reduced glutathione, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase and catalase.

Unsaturated lipids are particularly susceptible to oxidative modification and lipid peroxidation as a sensitive marker of oxidative stress (Lovell et al., 1995). Lipid peroxidation is the result of attack by free radicals on unsaturated fatty acids, such as linoleic acid and arachidonic acid, to generate highly reactive lipid peroxy radicals that initiate a cascade of reactions to further attack other unsaturated fatty acids. The chain reactions lead to the formation of breakdown product malondialdehyde other than 4-hydroxy-2, 3-nonenal (HNE), acrolein, and F2-isoprostanes. Thioisobutiric acid-reactive substances (TBARS, the most prevalent substrate of which is malondialdehyde) and
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We assessed the impact of antioxidants' treatment on the oxidative stress biomarkers in the L group rats with neurodegeneration induced by intracerebroventricularly administered STZ. The ICV-STZ infused rats had altered all the oxidative stress biomarkers as compared to sham. Further, ICV-STZ group pretreated with combined NCSe, antioxidants has significantly balanced all the oxidative stress biomarkers as compared to ICV- STZ group. The results reflect that oxidative stress is widespread, affecting measures of lipid peroxidation, reduced glutathione, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase and catalase.

Unsaturated lipids are particularly susceptible to oxidative modification and lipid peroxidation as a sensitive marker of oxidative stress (Lovell et al., 1995). Lipid peroxidation is the result of attack by free radicals on unsaturated fatty acids, such as linoleic acid and arachidonic acid, to generate highly reactive lipid peroxy radicals that initiate a cascade of reactions to further attack other unsaturated fatty acids. The chain reactions lead to the formation of breakdown products malondialdehyde other than 4-hydroxy-2, 3-nonenal (HNE), acrolein, and F2-isoprostanes. Thioarbituric acid-reactive substances (TBARS, the most prevalent substrate of which is malondialdehyde) and...
F2-isoprostanes are all increased in AD brains relative to age-matched controls (Arlt et al., 2002).

In our earlier findings (Ahmad et al., 2006) *Nordostachy jatamansi* (200, 400 and 600 mg/kg body weight, orally) was used as a neuroprotective agent in the Parkinsonism induced by 6-hydroxydopamine. Moreover, *Nordostachy jatamansi* (250 mg/kg body weight, orally) was also used as antioxidant in cerebral ischemic stroke (Salim et al., 2003). Earlier finding from our laboratory have shown the protective effect of crocetin (25, 50 and 75 μg/kg body weight) on hemi-Parkinson (Ahmad et al., 2005). However, selenium as sodium selenite (0.1, 0.2 and 0.3 mg/kg body weight) had protective effect in rat model of Parkinson’s disease (Zafar et al., 2003).

Our results of *Nordostachy jatamansi* on brain enzyme activity and its reported neurotrophic effects may partly explain the traditional use of this plant for improving cognition. Several plant species exhibited *in vitro* AChE inhibition further validating their use in traditional medicine. Those that did not exhibit activity in this investigation may act on a different mechanism related to their traditional use.

It is generally accepted that the clinical utility of a drug depends upon the delicate balance between the undesirable side effects and the desired protective effect. Most of the currently used antiepileptic drugs have neurotoxic effects, cognitive deficits and teratogenic potential, which limits their clinical utility (Trimble, 1987; Yerby, 1988; Meador et al., 1990). In our study, the combined drug NCSe showed negligible neurotoxicity at lower doses that protected the cognitive impairments and enhanced learning and memory.

In addition, the present study demonstrates that the combined extract might possess antioxidant property against the AD model. However, this extract failed to abolish the tonic extensor phase completely. By and large, the traditional system of medicine is slow acting as compared to the modern synthetic drugs because they are administered as crude preparations. However, in conjunction with active constituents and synthetic drugs, they may protect the neurodegeneration of the brain. The synergistic effects of the combined drugs scavenge the free radicals very fast as compared to single drug alone. The goal of polytherapy would be to have better effect than monotherapy and less toxic than standard drugs as the combined drugs are effective at much lower concentrations than the individual drugs alone. The research in the above direction will also throw an insight into the possible mechanism of action of different drug combinations. It is well established that ICV-STZ decreases the local glucose, ATP, acetylcholine esterase (Plaschke and Hoyer, 1993; Hoyer et al., 1996; Hoyer, 2000) and alteration of calcium influx and efflux in the AD patients (Watt, 1996; Mattson and Chan, 2001). However, very few reports are available on the mechanisms by which the herbs or herbal combinations with synthetic drugs increase the therapeutic potency or reduce the adverse effects of STZ neurotoxicity in the brain. Our earlier study has clearly shown that combination of many constituents (Khan et al., 2006) protects cognitive impairments in ICV-STZ infused rat model and *Nordostachy jatamansi* has an influence on 6-hydroxypamine induced parkinsonism in rats (Ahmad et al., 2006).

The protection offered by either *Nordostachy jatamansi* (Ahmad et al., 2006), crocetin (Ahmad et al., 2005) or selenium (Zafar et al., 2003) in the 6-OHD model of Parkinsonism compares very favorably with the most effective neuroprotective agents. It is difficult to speculate the additive effects of the combined drugs because no data are available to explain the phenomena. It is, therefore, possible that co-administration of NCSe modulates ICV-STZ induced oxidative stress-related cognitive impairments, resulting in neuroprotection. However, other unidentified biologic effects of the two and more drug combinations are probable. It is possible that *Nordostachy jatamansi* and crocetin somehow interact with Se, thereby enhancing the protective effect observed with lower Se dose and eliminates the damaging effect of neuron
and maintains the integrity of the whole neurotoxic and excitotoxic cascades leading to cell death.

In this study, we have demonstrated the potent synergistic action of *Nardostachys jatamansi* extract and crocetin in conjunction with selenium in the increased percentage of protection against cognitive impairments. Moreover, many investigators have suggested that the experimental protective index could better express the clinical utility between the undesirable side effects and the desired drug effects. Our study showed a significant increase in the protective index, indicating a pharmacodynamic interaction of the combined drug regime but do not rule out the contribution of pharmacokinetic interactions.

In conclusion, the combined-drugs study underscores the significance of the synergistic effect of *Nardostachys jatamansi*, crocetin in conjunction with selenium where the dose of the selenium is at lower concentration. The behavioral modifications suggested the possible potential activities of the NCSe on central nervous system. This corroborates with our previous findings that showed the changes in the brain enzymes activities. The increase in the protective index with the combined drugs suggests that, this could be of particular interest in cognitive impairments especially Alzheimer's disease type of dementia that is controlled only by the lower doses of antioxidants drugs currently used. However, further research is necessary to determine the components involved and their mechanism of action in bringing about the desirable pharmacological effect.

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