Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Neuroprotective effect of selenium in rats subjected to chronic intermittent cold and immobilization stress: The role of phosphorylated tau

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ARTICLE INFO

Article type:
Original

Article history:
Received: Dec 2, 2025
Accepted: May 25, 2026

Keywords:
Chronic stress
Cognitive impairment
Hippocampus
Neuroinflammation
Oxidative stress
p-tau
Rat
Selenium

ABSTRACT

Objective(s): The current study aimed to investigate the potential neuroprotective effect of selenium against cognitive dysfunction in rats subjected to chronic intermittent stress.

Materials and Methods: Ninety adult male Wistar rats were randomly assigned into five groups (n=18): (1) N: non-stressed group; (2) CS: cold-stressed non-treated group; (3) IS: immobilized-stressed non-treated group; (4) CS/Se: cold-stressed/ selenium-treated group; and (5) IS/Se: immobilized-stressed/ selenium-treated group. Memory and learning were assessed using the Morris Water Maze, novel object recognition, and Y-maze tests. After completion of neurobehavioral tests, fasting retro-orbital blood samples were collected from all rats. Stress hormones, serum sodium and potassium, oxidative stress markers (malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione peroxidase (GPx), and thioredoxin reductase (TrxR)), and the proinflammatory markers (tumor necrosis factor-alpha (TNF-α), intercellular adhesion molecule-1 (ICAM-1)) were measured. Histopathological examination of the hippocampus and immunohistochemistry of p-tau were performed.

Results: The results indicated that memory and learning were impaired by chronic intermittent stress. This was associated with histopathological changes of the hippocampus and increased the expression of p-tau. Selenium administration improved the cognitive dysfunction as indicated by behavioral tests. Also, it reduced the levels of stress hormones, sodium, MDA, and the proinflammatory markers. It significantly enhanced the antioxidant enzyme activities and increased the potassium level. In addition, it improved the histopathological changes of the hippocampus and decreased the expression of p-tau.

Conclusion: Coadministration of selenium to rats subjected to chronic stress improved cognitive dysfunction by its antioxidant and anti-inflammatory effects, as well as via downr-egulation of p-tau in the hippocampus.

▶Please cite this article as:
Abdallah MA, Abdel-Razek HAD; Saleh SA, Elseadawy RSA, Kora MA, Younis AG, Salem HR. Neuroprotective effect of selenium in rats subjected to chronic intermittent cold and immobilization stress: The role of phosphorylated tau. Iran J Basic Med Sci 2026; 29: 1440-1447. doi: https://dx.doi.org/10.22038/ijbms.2026.93270.20117

Introduction

Stress is a condition of threatened homeostasis in response to extrinsic or intrinsic stressors. It is counteracted by physiological, neuroendocrine, and behavioral responses aimed at preserving or reestablishing the set point of body control systems (1). However, excessive prolonged stress has a negative impact on physical and mental health. These effects are mainly due to overactivation of the sympathoadrenal medullary axis (SMA) and the hypothalamic-pituitary-adrenal axis (HPAA)(2).

Chronic stress can impair cognitive function, hasten neurodegeneration, and increase the risk of Alzheimer's disease. Stress induces cognitive dysfunction through activation of apoptosis, endoplasmic reticulum stress, oxidative stress, and neurotransmitter imbalance (3). Hyperphosphorylation of the tau protein (p-tau) is also involved in stress-induced cognitive dysfunction. P-tau is essential for stabilizing microtubules and preserving the normal morphology of nerve cells (4).

Recently, natural antioxidants have gained interest owing to their protective role against oxidative free-radical-mediated pathophysiological effects (5). Selenium, a nutritionally essential trace element, is a main component of several antioxidant enzymes with strong antioxidant capacity. It can guard the brain from oxidative damage in several neurodegenerative models (6). Selenium is naturally found in water, soil, and food (7).

Several studies have demonstrated the neuroprotective effect of selenium across various experimental models (8-10). However, the underlying mechanisms are still unclear,

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and its efficacy against cognitive dysfunction induced by cold stress and the underlying modulation of p-tau in this specific model had not been previously explored. Here, this study aimed to investigate and compare the potential neuroprotective effect of selenium against cognitive dysfunction in rats subjected to chronic cold stress versus chronic immobilization stress. The study was focused on the effects on markers of oxidative/antioxidant status, proinflammatory cytokines, and the assessment of p-tau in the hippocampus.

Materials and Methods

Ethics statement

The experimental protocol was approved by the local ethics committee of the Faculty of Medicine, Menoufia University, Egypt (IRP approval number: 6/2022 PHYS 39). Rats were treated in accordance with Guide for the Care and Use of Laboratory Animals (18th edition, National Academies Press).

Animals

Ninety adult male Wistar rats aged 3-6 months and weighing 200-250 g were employed in the study. Rats were kept in fully ventilated cages (6/cage), measuring 60 L x 60 W x 25 H cm. They had unrestricted access to food and water, unless stressors were applied. Before being used in any experimental procedures, they were given a week to acclimate to the housing facility. All experiments were conducted between 9 a.m. and 3 p.m.

Experimental design

The animals were randomly allocated into 5 groups (n=18). (1) Non-stressed (N) group: rats were left undisturbed in their home cages during the experimental period. They were given 1 ml of 0.9% NaCl, the vehicle of sodium selenite, daily by oral gavage for 2 weeks. (2) Cold-stressed non-treated(CS) group: rats were subjected to chronic intermittent cold stress (CICS) 6 h/day at 4 ˚C for 2 weeks (11). (3) Immobilized-stressed non-treated(IS) group: rats were subjected to chronic intermittent immobilization stress (CIIS) 6 h/day for 2 weeks (12). (4) Cold-stressed/selenium-treated (CS/Se) group: rats received sodium selenite (Sigma Chemical Co., USA) dissolved in 0.9% NaCl at a dose of 0.35 mg/kg, once daily by oral gavage, 1 hour prior to exposure to CICS sessions for 2 weeks. The therapeutic dose of selenium was based on previous studies (13, 14). (5) Immobilized-stressed/selenium-treated (IS/Se) group: rats received sodium selenite dissolved in 0.9% NaCl at a dose of 0.35 mg/kg/d once daily by oral gavage, 1 hour prior to exposure to CIIS sessions for 2 weeks. The therapeutic dose of selenium was based on previous studies (13, 14).

At the end of 2 weeks, rats in each group were randomly divided into 3 subgroups (n=6) to assess different aspects of cognitive function: Subgroup A: spatial reference memory and learning using the Morris Water Maze (MWM) test. Subgroup B: non-spatial memory using the novel object recognition (NOR) test. Subgroup C: spatial working memory using the Y-maze test. All neurobehavioral tests were conducted by two independent researchers who were blinded to the experimental group assignments.

The day after completion of neurobehavioral tests, fasting retro-orbital blood samples were collected from all rats, and serum was separated and stored at -80˚ until use.

Serum samples were used for measurement of stress hormones (corticosterone, adrenaline, and noradrenaline), sodium and potassium, oxidative stress markers (malondialdehyde (MDA), total antioxidant capacity (TAC)), and the proinflammatory markers (tumor necrosis factor-alpha (TNF- α) and intercellular adhesion molecule-1 (ICAM-1)).

Lastly, animals were sacrificed by cervical elongation and dislocation, and the brains were excised and divided into 2 halves for exposure and isolation of the hippocampus. The right half of the hippocampus was homogenized and used to measure the brain activity of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). The left half of the hippocampus was prepared for histopathological and immunohistochemical studies.

Homogenization of hippocampal tissue

Immediately after dissection, the brain was rinsed in ice-cold phosphate-buffered saline solution, pH 7.4, blotted with filter paper, and hippocampi were separated and homogenized using a tissue homogenizer in cold buffer (5 ml/g tissue).

Biochemical analysis

Serum levels of corticosterone, epinephrine, norepinephrine, TNF-α, and ICAM-1 were measured using ELISA kits (Cat. ab323743, ab287788, ab287789, Cat. Ab208348, and b171123, Abcam, respectively). Serum sodium, potassium, MDA, and TAC were measured by colorimetric methods using diagnostic kits (Bio-diagnostic, Egypt). The activities of GPx and TrxR enzymes were measured in hippocampal tissues using colorimetric kits (Cat. ab102530 and ab83463, respectively). All procedures were done according to the manufacturer's instructions.

Neurobehavioral tests

Morris water maze (MWM)

Spatial memory and learning were assessed by MWM as previously described (15). Rats in each subgroup were trained on the MWM task for 4 consecutive days.

Rats were trained for 4 days to search for a hidden, submerged platform in a water pool. Four 60-second trials were performed each day. Four fixed start locations (N, S, E, W) were used daily. The sequences were changed every day. The platform's position was fixed for all training days. The probe trial was done 1 hour after the last training trial on the fourth day. The platform was removed, and the rat was placed in a novel start position in MWM. The trial was terminated if the rat spent 5 seconds in the target zone.

Novel object recognition (NOR) test

Non-spatial long-term memory was assessed using the NOR test, as previously described (16). The NOR apparatus is a square open box (35×40×43 cm) made of dark brown wood. First, the rats were habituated for 5 min in the empty box. After 2 hr, a learning trial was conducted using 2 similar familiar objects (FO) for 5 min. A test trial was conducted 24 h later, during which one familiar object was replaced by a new object (NO), and exploration was allowed for 5 min. The time spent near each object was recorded for 5 min. Exploration behaviors include sniffing, orienting toward the object, or placing the nose within 1 cm of the object. The

objects and box were cleaned with 50% ethanol to eliminate any residual odors after each session. Discrimination index (D.I.) was calculated as ((NO exploration time - FO exploration time)/total exploration time). Recognition index (R.I.) was calculated as ((NO exploration time/total exploration time of FO and NO) X 100).

Y-maze test

Spatial working memory was assessed using a Y-maze, as previously described (17). The Y-maze consisted of 3 labeled arms (40 cm long x 13 cm wide x 12 cm high) radiating from a central point at 120° angles to one another. Each rat was initially placed in one arm, and the sequence of arm entries and the total number of entries were recorded over an 8-minute period. The percentage of spontaneous alternation was calculated as follows: number of successful alternations/ (total arm entries - 2) x 100.

Histopathological examination

The excised hippocampal tissue from each rat was fixed and preserved in 10% neutral-buffered formalin. Samples were then dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. 4-µm-thick sections were prepared for hematoxylin and eosin (H&E) staining of the hippocampus. A blinded, expert pathologist examined the sections under a light microscope.

Immunohistochemistry of p-tau

Slides were deparaffinized in xylene and rehydrated through an ascending alcohol series. Antigen retrieval and blocking of endogenous peroxidase were performed by boiling in citrate-buffered saline (pH 6) and incubation in 3% H_2O_2, respectively. Slides were incubated with the primary antibody (rabbit polyclonal antibody against Phospho-Tau-T231 pAb; Catalog No. AP0401, ABclonal USA) overnight at room temperature, followed by incubation with the secondary antibody. DAB and Mayer's hematoxylin were used as the chromogenic substrate and counterstain, respectively. The specificity of the immune reaction was controlled by omitting the primary antisera. The immunoreactive score (IRS) was calculated (18).

Statistical analysis

Statistical analysis was performed using SPSS (version 22; SPSS Inc., Chicago, IL, USA). A one-way ANOVA was used to determine statistically significant differences among the groups, followed by a post hoc Tukey test. A *P*-value< 0.05 was considered significant. All results are presented as mean±S.D.

Results

Assessment of stress hormones

Rats subjected to chronic intermittent stress in the CS and IS groups showed significant elevations in serum corticosterone, epinephrine, and norepinephrine compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly lowered these values in the CS/Se and IS/Se groups compared with the untreated CS and IS groups, respectively (*P*=0.001). The IS and IS/Se groups showed significantly higher values than the CS and CS/Se groups, respectively (*P*=0.001). The values in the CS/Se and IS/Se groups were significantly higher than those in the N group (*P*=0.001)(Table 1).

Assessment of serum sodium and potassium

Rats subjected to chronic stress in the CS and IS groups showed significant elevations in serum sodium and significant declines in serum potassium levels compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly lowered serum sodium and increased serum potassium in CS/Se and IS/Se groups compared with non-treated CS and IS groups, respectively (*P*=0.001). IS and IS/Se groups showed significantly higher serum sodium and significantly lower serum potassium than CS and CS/Se groups, respectively (*P*=0.001). Serum sodium was significantly higher, and serum potassium was significantly lower in CS/Se and IS/Se groups than in the N group (*P*=0.001)(Table 1).

Evaluation of oxidative/antioxidant status

Rats subjected to chronic stress in the CS and IS groups showed a significant increase in serum MDA compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly reduced MDA levels in the CS/Se and IS/Se groups compared with the untreated CS and IS groups, respectively (*P*=0.001). The IS and IS/Se groups showed significantly higher values than the CS and CS/Se groups, respectively (*P*=0.001). The values in the CS/Se and IS/Se groups were significantly higher than those in the N group (*P*=0.001)(Table 2).

Rats subjected to chronic stress in the CS and IS groups showed a significant decline in serum TAC, GPx, and TrxR

Table 1. Effect of chronic intermittent stress and selenium administration on serum levels of corticosterone, epinephrine, norepinephrine, sodium, and potassium in the studied rat groups

	N	CS	IS	CS/Se	IS/Se
Serum corticosterone (ng/ml)	36.4±3.74	78.4±2.56^{a}	129.5±3.42^{ab}	60.7±3.47^{ac}	97.8±2.62^{abc}
Serum epinephrine (pg/ml)	43.5±5.47	91.5±5.24^{a}	150.3±9.5^{ab}	67.5±70^{ac}	119.7±6.89^{abc}
Serum norepinephrine (pg/ml)	193.4±7.7	347.2±6.3^{a}	573.8±5.7^{ab}	271.2±5.03^{ac}	440.3±3.40^{abc}
Serum Na^{+} (mmol/l)	135.9±1.02	154.9±0.7^{a}	165.1±0.7^{ab}	144.5±0.50^{ac}	159.6±0.6^{abc}
Serum K^{+} (mmol/l)	5.02±0.12	3.95±0.12^{a}	3.58±0.03^{ab}	4.65±0.04^{ac}	4.03±0.07^{abc}

Data are expressed as mean±SD (n=18)
[a] significant vs N group, [b] significant vs equivalent cold-stressed group, [c] significant vs equivalent stressed non-treated group
N, non-stressed group; CS, cold-stressed non-treated group; IS, immobilized-stressed non-treated group; CS/Se, cold-stressed/selenium-treated group; IS/Se, immobilized-stressed/selenium-treated group

Table 2. Effect of chronic intermittent stress and selenium administration on rat oxidative stress markers (malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione peroxidase (GPx), and thioredoxin reductase (TrxR)), and the proinflammatory markers (tumor necrosis factor-alpha (TNF- α), intercellular adhesion molecule-1 (ICAM-1))

	N	CS	IS	CS/Se	IS/Se
Serum MDA (nmol/ml)	5.19±0.25	13.77±0.63[a]	20.89±0.52[ab]	8.82±0.41[ac]	11.8±0.38[abc]
Serum TAC (mmol/l)	3.19±0.27	1.5±0.06[a]	0.55±0.05[ab]	2.96±0.19[c]	2.01±0.12[abc]
GPx (IU/g of tissue)	29.07±0.69	24.57±0.26[a]	15.8±0.35[ab]	28.47±0.27[c]	20.06±0.35[abc]
TrxR (nmol/min/mg protein)	11.03±0.32	8.15±0.27[a]	6.95±0.34[ab]	10.52±0.74[c]	8.05±0.20[abc]
Serum TNF- α (pg/ml)	44.5±2.26	66.17±2.99[a]	94.5±2.17[ab]	52.8±2.64[ac]	81±1.67[abc]
Serum ICAM-1 (pg/ml)	84.8±1.47	117.3±1.86[a]	144.5±1.38[ab]	103.6±1.86[ac]	134.5±2.66[abc]

Data are expressed as mean±SD (n=18)
[a] significant vs N group, [b] significant vs equivalent cold-stressed group, [c] significant vs equivalent stressed non-treated group
N, non-stressed group; CS, cold-stressed non-treated group; IS, immobilized-stressed non-treated group; CS/Se, cold-stressed/selenium-treated group; IS/Se, immobilized-stressed/selenium-treated group

in hippocampal tissue compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly increased these values in the CS/Se and IS/Se groups compared with the untreated CS and IS groups, respectively (*P*=0.001). The IS and IS/Se groups showed significantly lower values than the CS and CS/Se groups, respectively (*P*=0.001). In the IS/Se group, serum TAC and GPx and TrxR in hippocampal tissue were significantly lower than in the N group (*P*=0.001), whereas there was no significant difference between the CS/Se and N groups (p=0.148, p=0.121, and p=0.244, respectively)(Table 2).

Estimation of proinflammatory cytokines

Rats subjected to chronic stress in the CS and IS groups showed significant increases in serum TNF-α and ICAM-1 compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly reduced these values in the CS/Se and IS/Se groups compared with the untreated CS and IS groups, respectively (*P*=0.001). The IS and IS/Se groups showed significantly higher values than the CS and CS/Se groups, respectively (*P*=0.001). The values in the CS/Se and IS/Se groups were significantly higher than those in the N group (*P*=0.001)(Table 2).

Assessment of spatial memory and learning (MWM)

Rats subjected to chronic stress in the CS and IS groups showed a significant increase in escape latency on the probe trial in the MWM compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly reduced the values in the CS/Se and IS/Se groups compared with the non-treated CS and IS groups, respectively (*P*=0.001). IS and IS/Se groups showed significantly higher values than CS and CS/Se groups, respectively (*P*=0.001). The values in the CS/Se and IS/Se groups were significantly higher than the N group (*P*=0.001)(Figure 1).

Assessment of non-spatial long-term memory (NOR)

Rats subjected to chronic stress in CS and IS groups showed a significant decrease in DI and RI compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly increased the values in the CS/Se and IS/Se groups compared with the non-treated CS and IS groups, respectively (*P*=0.001). IS and IS/Se groups showed significantly lower values than CS and CS/Se groups, respectively (*P*=0.001). The values in the CS/Se and Se/IS groups were significantly lower than the N group (*P*=0.001) (Figure 2).

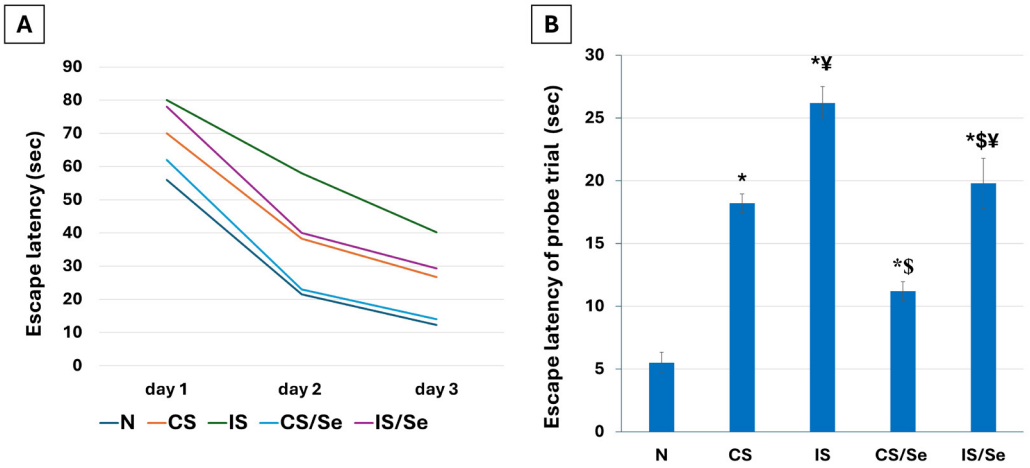

Figure 1. Morris water maze analysis in rats
Data are expressed as mean±SD (n=6).* Significant vs N group, ¥ significant vs equivalent cold-stressed group, $ significant vs equivalent stressed non-treated group
N, non-stressed group; CS, cold-stressed non-treated group; IS, immobilized-stressed non- treated group; CS/Se, cold-stressed/selenium-treated group; IS/Se, immobilized-stressed/selenium-treated group

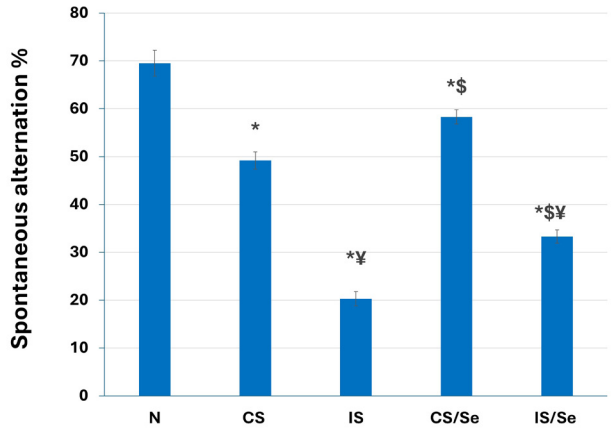

Figure 2. Analysis of Y-Maze in rats
Data are expressed as mean±SD (n=6).* Significant vs N group, ¥ significant vs equivalent cold-stressed group, $ significant vs equivalent stressed non-treated group N, non-stressed group; CS, cold-stressed non-treated group; IS, immobilized-stressed non-treated group; CS/Se, cold-stressed/selenium-treated group; IS/Se, immobilized-stressed/selenium-treated group

Assessment of spatial working memory (Y-maze test)

Rats subjected to chronic stress in the CS and IS groups showed a significant decrease in spontaneous alternation compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly increased the values in the CS/Se and IS/Se groups compared with the untreated CS and IS groups, respectively (*P*=0.001). The IS and IS/Se groups showed significantly lower values than the CS and CS/Se groups, respectively (*P*=0.001). The values in the CS/Se and IS/Se groups were significantly lower than those in the N group (*P*=0.001)(Figure 3).

Histopathologic examination

H&E examination revealed a normal histologic appearance of the hippocampi in the N group. Rats subjected to CICS showed mild degenerative changes, mainly in the pyramidal cell layer. Rats subjected to CIIS showed marked degenerative changes, mainly in the pyramidal cell layer. Most cells were surrounded by a peripheral halo. The nerve fibers were widely separated. Treatment with selenium showed good improvement in histopathological changes in the CS/Se group and moderate improvement in the IS/Se group compared with non-treated rats (Figure 4).

Immunohistochemistry of p-tau

Rats subjected to chronic stress in CS and IS groups showed significant elevations in IRS of p-tau compared with non-stressed rats (*P*=0.001). Treatment with selenium significantly lowered the values in CS/Se and IS/Se groups compared with non-treated CS and IS groups, respectively

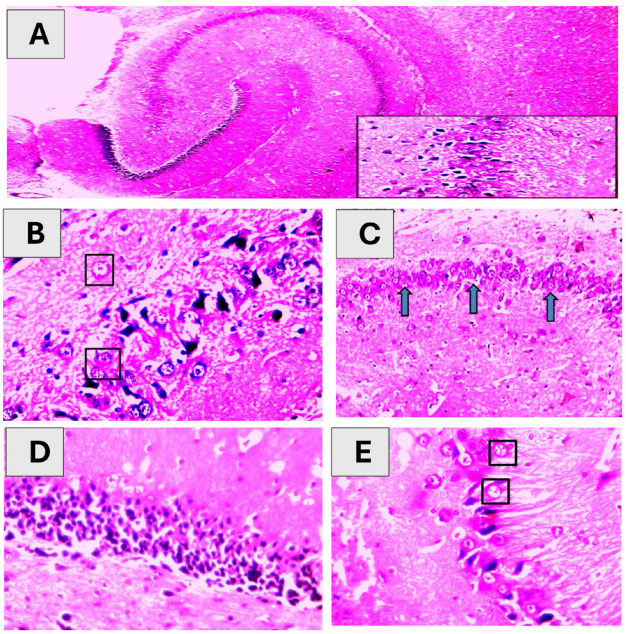

Figure 4. Light H&E-stained micrographs of the rat hippocampus
(A) Non-stressed group showing intact histological structure. The inset at the lower left corner highlights the pyramidal cell layer showing large, rounded vesicular nuclei and prominent nucleoli (H&E x100 and inset x400). (B) Cold-stressed non-treated group showing mild degeneration mainly in the pyramidal cell layer (squares). Some pyramidal cells showed degenerative changes, while others had acidophilic cytoplasm and vesicular nuclei. (H&E x400). (C) The immobilized-stressed, untreated group showed marked degeneration (arrows), mainly in the pyramidal cell layer. Most of the cells appeared shrunken with darkly stained cytoplasm and deeply pyknotic nuclei. Most cells were surrounded by a peripheral halo. The nerve fibers were widely separated. (H&E x400). (D) Cold-stressed/selenium-treated group showing mild improvement (H&E x400). (E) Immobilized-stressed/selenium-treated group showing moderate improvement (H&E x400)

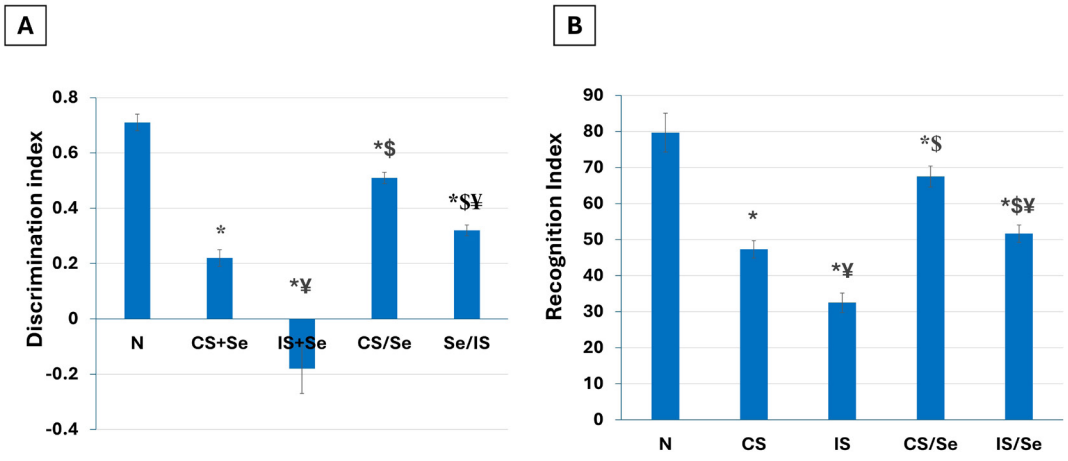

Figure 3. Analysis of rat novel object recognition test
Data are expressed as mean±SD (n=6). * Significant vs N group, ¥ significant vs equivalent cold-stressed group, $ significant vs equivalent stressed non-treated group
N, non-stressed group; CS, cold-stressed non-treated group; IS, immobilized-stressed non-treated group; CS/Se, cold-stressed/selenium-treated group; IS/Se, immobilized-stressed/selenium-treated group

(*P*=0.001). IS and IS/Se groups showed significantly higher values than the CS and CS/Se groups (*P*=0.001 and *P*=0.005, respectively). The value in the IS/Se group was significantly higher than the N group (*P*=0.001), while there was an insignificant change between the CS/Se and N groups (p=0.117)(Figure 5).

Discussion

Chronic intermittent stress impairs cognition by elevating oxidative stress parameters. Consequently, selenium, a natural antioxidant, can afford a neuroprotective effect (19). In the present investigation, CICS and CIIS, administered for 6 hr daily for 14 days, induced a significant decline in cognitive functions, including spatial memory and learning, non-spatial memory, and working memory, as indicated by neurobehavioral test results. Impaired memory under chronic stress is consistent with previous studies (20-22).

The mechanism of stress-induced cognitive dysfunction is multifactorial. Chronic stress overactivates the SMA and HPAA, leading to significant elevation of stress hormones, as confirmed in this study, indicating successful establishment of a chronic stress model (23). Also, a disturbed oxidative/antioxidant status was observed, as indicated by elevated MDA and reduced antioxidant enzyme activity. The diminished activity of antioxidant enzymes in the brain could be explained by the ability of glucocorticoids to down-regulate numerous types of anti-oxidant enzymes, including GPx, which supports the current results (19). Hippocampal oxidative stress may also explain the decline in cognitive function in stressed, untreated groups (24).

In addition to oxidative damage, chronic stress can induce neuroinflammation and promote neurodegenerative diseases. Neuroinflammation is mediated by the release of inflammatory cytokines from the periphery or those locally produced in the brain (25). Stress-induced inflammatory status was evidenced by significant elevation of TNF-α and ICAM-1 in stressed rats compared with non-stressed rats. Lu *et al.* (26) found that chronic psychological stress significantly elevated the expression of proinflammatory cytokines TNF-α and ICAM-1, inducing vascular inflammation in the aortic walls in rabbits, which agrees with the current results. ICAM-1 has been proposed as a biomarker of endothelial dysfunction and its associated cognitive decline in Alzheimer's disease (27).

The impaired cognition in stressed rats was associated with degenerative changes in the hippocampus. The current results agree with previous investigations that confirmed the harmful effects of chronic stress on hippocampal structure and function (28). The hippocampus is one of the brain regions most vulnerable to chronic stress-induced injury due to the high expression of mineralocorticoid and glucocorticoid receptors (29). Also, oxidative stress is implicated in stress-induced hippocampal damage. Al-Sowayan *et al.* (20) reported that chronic immobilization stress significantly increased MDA levels and decreased antioxidant enzyme activity, supporting the current results. Chronic cold stress could induce hippocampal apoptosis due to cellular oxidative stress and inflammatory injury (30).

Interestingly, stressed rats showed increased p-tau expression in the hippocampus compared with non-stressed rats. In line with our results, chronic restraint stress significantly increased p-tau immunoreactivity in the hippocampus of rats (31). P-tau is a biomarker for neurodegenerative diseases such as Alzheimer's disease. Hyperphosphorylation of tau forms aggregates that impair axonal or dendritic transport in neurons (32). Increased p-tau may be related to elevated glucocorticoid

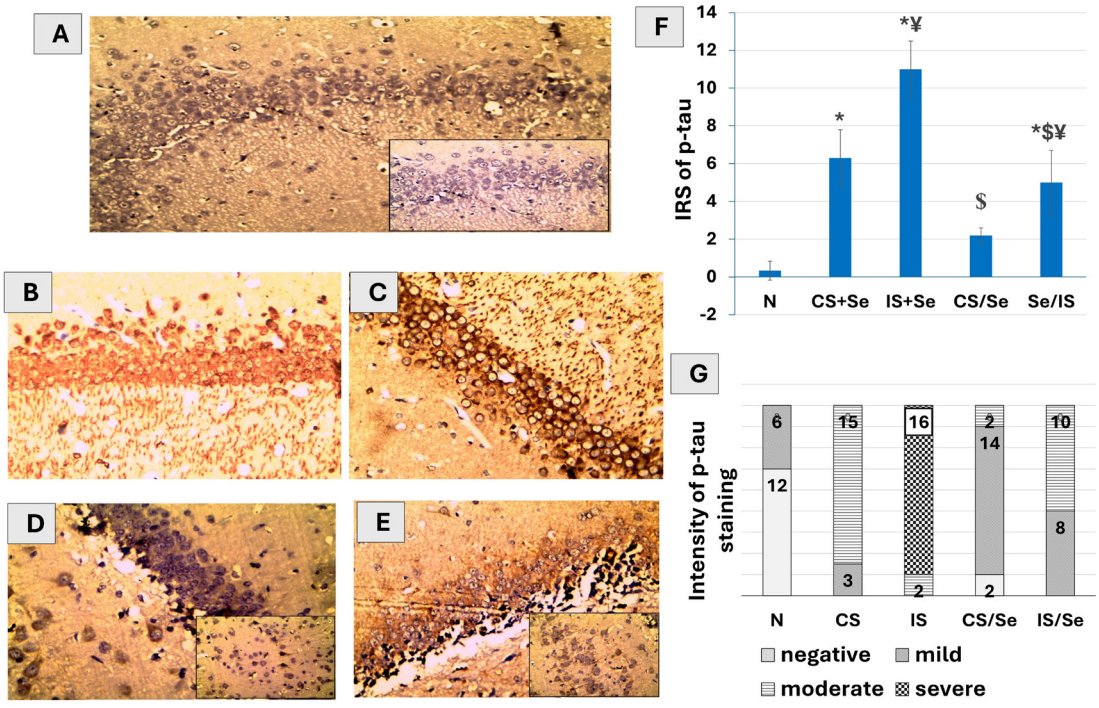

Figure 5. Tau protein (P-tau) immunohistochemistry in rat hippocampus
(A) non-stressed, (B) cold-stressed non-treated, (C) immobilized-stressed non-treated, (D) cold-stressed/selenium-treated, (E) immobilized-stressed/selenium-treated groups (IHC original magnification x400). (F) Immunoreactive score (IRS) of p-tau immunostaining and (G) Intensity of p-tau immunostaining in the studied groups

levels, which augment tau hyperphosphorylation and aggregation (33). Oxidative stress also directly promotes tau hyperphosphorylation. MDA levels are increased in tau pathology (34).

The present investigation showed that CIIS caused more cognitive dysfunction than CICS. This can be explained by the greater elevation of stress hormones, oxidative stress, and inflammatory biomarkers in the IS group than in the CS group. These results agree with a previous study (35).

Our findings reveal that selenium administration one hour before stress sessions for 2 weeks significantly improved performance in neurobehavioral tests. The neuroprotective effect of selenium against memory deficits was supported by previous studies (17, 36). Also, selenium administration to stressed rats significantly lowered corticosterone levels. This can explain the improvement in cognitive brain functions under stress (37).

The improvement in cognitive deficits in selenium-treated rats was evidenced by reduced histopathological changes in the hippocampus. There was a decrease in degenerative changes induced by chronic stress exposure. This is consistent with (8), which confirmed the favorable effects of selenium on the hippocampus.

The improved cognitive dysfunction in selenium-treated groups could be explained by its antioxidant and anti-inflammatory effects, as proved by the biochemical results.

Selenium supplementation in rats subjected to CICS and CIIS was highly effective in preventing oxidative stress and neuroinflammation. The antioxidant effect of selenium minimizes oxidative damage in memory-related brain areas, such as the hippocampus (9). Selenium-containing antioxidant enzymes, such as GPx and TrxR, directly suppress oxidative stress, as reported previously (38). Selenium can also scavenge reactive oxygen species from brain tissue, preventing lipid peroxidation. Moreover, selenium derivatives, acting as antioxidants, can directly affect synaptic transmission and plasticity, improving memory and learning (39). The anti-inflammatory action of selenium can be explained by its ability to down-regulate macrophage signaling pathways, thus inhibiting microglial release of pro-inflammatory cytokines (40).

Notably, we found that selenium supplementation in stressed rats significantly reduced hippocampal p-tau levels. Consistent with this, selenium improved tau pathology in an Alzheimer's disease rat model (39). Selenoprotein W, a selenium-rich antioxidant protein implicated in selenium transport, binds to tau, thereby reducing tau accumulation. Selenoprotein W is sensitive to selenium levels. Its deficiency may lead to synaptic defects, tau pathology, and worsened long-term potentiation, resulting in memory deficits in mice (41).

Conclusion

Selenium exhibits neuroprotective effects against cognitive dysfunction in rats exposed to chronic intermittent stress through antioxidant and anti-inflammatory mechanisms, as well as by down-regulating p-tau expression, collectively preserving hippocampal neuronal health. The current results suggest that selenium could be a protective agent for preventing stress-induced cognitive dysfunction. Nevertheless, further research is needed to fully elucidate the specific molecular pathways and to evaluate the translational potential of these findings in clinical settings.

While these results suggest that selenium holds promise as a therapeutic strategy, further research is required to evaluate the translational potential and safety of these findings in a clinical setting. (Changed "therapeutic agent" to "potential therapeutic strategy").

Acknowledgment

The authors extend their appreciation to the central laboratory at the Faculty of Medicine, Menoufia University, Egypt, for providing facilities.

Authors' Contributions

SA S and HAD A designed the experiments, supervised, directed, and managed the study; MA A, AG Y, MA K, and HR S performed experiments and collected data; MA A, AG Y, and HR S analyzed the results; MA A, AG Y, RSA E, and HR S prepared the manuscript; SA S and HAD A approved the final version to be published.

Conflicts of Interest

None.

Declaration

The authors declare that no artificial intelligence tools were used in preparing, collecting data for, or analyzing this manuscript. All content is original and has been developed solely by the authors.

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