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Nano-selenium and the SRC family kinases pathway: Redefining gene expression dynamics in major depressive disorder based on a randomized controlled trial

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Morvarid Noormohammadi[1], Farnaz Etesam[2,3], Ali Amini[4], Pegah Khosravian[5], Morteza Mohammadzadeh[6], Farzad Shidfar[1,7]

[1] Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[2] Psychosomatic Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Psychiatry, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Hematology and Blood Banking, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
[5] Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[6] Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[7] Nutritional Sciences Research Center, Iran University of Medical Sciences, Tehran, Iran

Keywords
Major Depressive Disorder; Nano-Selenium; SRC Gene; Randomized Clinical Trial; Gene Expression

Abstract

Background: Major depressive disorder (MDD) is a prevalent psychiatric condition. Dysregulation of signaling pathways interacting with SRC family kinases has been implicated in the pathophysiology of MDD through inflammation. Nano-selenium, a nanoscale form of selenium with enhanced bioavailability, has the potential to modulate oxidative stress and inflammation, which are implicated in MDD. To the best of our knowledge, in this study, for the first time we aimed to examine whether nano-selenium supplementation would decrease c-SRC gene expression.

Methods: This triple-blind, randomized, placebo-controlled clinical trial was conducted at Imam Khomeini Hospital Complex, Tehran, Iran.

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Corresponding Author: Farzad Shidfar
Email: farzadshidfar@yahoo.com

Using block randomization, fifty participants diagnosed with MDD per Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) criteria were randomly assigned to receive nano-selenium (55 µg/day, n = 25) or placebo (n = 25) for 12 weeks alongside sertraline (50 mg/day). c-SRC gene expression was assessed using real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) from peripheral blood samples collected at baseline and after 12 weeks.

Results: Twenty-one participants in each group completed the study, with an 84% retention rate in both groups. No serious adverse events were reported. There was no significant difference between groups at baseline. Post-intervention, c-SRC gene expression levels decreased in both the nano-selenium and placebo groups [median change (Q1, Q3): -0.0031, (-0.0065, -0.0005) vs. -0.0021 (-0.0085, 0), respectively; P < 0.05]; however, no significant differences were observed between the two groups (P = 0.606).

Conclusion: Nano-selenium supplementation did not significantly modulate c-SRC gene expression. Limitations included a short duration. Future studies should explore alternative molecular pathways, higher supplementation doses, and treatment-naïve populations to better understand nano-selenium's therapeutic potential in MDD.

Introduction

Major depressive disorder (MDD) is a debilitating psychiatric condition characterized by persistent low mood, diminished interest in activities, cognitive impairments, and a range of somatic symptoms.[1] It affects more than 280 million people worldwide and is a leading cause of disability.[2] Despite advancements in pharmacological and psychological therapies, such as sertraline, MDD remains a significant global health challenge. Up to 30%-50% of patients with MDD do not respond adequately to first-line antidepressants like selective serotonin reuptake inhibitors (SSRIs), including sertraline.[3] Many patients require multiple trials of medications, often without achieving full remission. Besides, most antidepressants take weeks to months to show their full therapeutic effects, leaving patients vulnerable to prolonged distress and increased suicide risk.[4] Additionally, many patients experience significant side effects from SSRIs, such as weight gain, sexual dysfunction, and emotional blunting, which lead to poor adherence and treatment discontinuation.[5] Finally, current antidepressants primarily target neurotransmitters (serotonin, dopamine, norepinephrine), but they do not address underlying inflammatory mechanisms involved in many cases of MDD.[6] These constraints have driven a heightened focus on discovering new therapeutic targets and mechanisms to enhance the treatment of MDD.

Chronic inflammation and immune system dysregulation are increasingly recognized as contributing factors in MDD.[7] Elevated levels of pro-inflammatory cytokines [e.g., interleukin 6 (IL-6), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α)] are commonly observed in patients with MDD.[7] Many of these cytokines signal through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, suggesting that dysregulation of this pathway may contribute to depressive symptoms.[8] SRC family kinases play a crucial role in the regulation of the JAK/STAT signaling pathway. SRC kinases act as upstream regulators of JAK/STAT activation.[9] The phosphorylated Janus kinases (JAKs) lead to the activation of signal transducer and activator of transcription (STAT) proteins, which then translocate to the nucleus to regulate gene expression.[10] In addition, increased SRC kinases activity has been observed in neuroinflammatory conditions and may contribute to the heightened immune response seen in MDD.[11-13] Besides, SRC kinases interact with N-methyl-D-aspartate (NMDA) receptors, modulating glutamatergic neurotransmission.[14] Dysfunction of NMDA receptors has been implicated in depression, and SRC-mediated NMDA receptor modulation could influence mood regulation.[15] Moreover, SRC kinases help regulate the permeability of the blood-brain barrier (BBB). In MDD, increased inflammation can lead to BBB disruption, allowing peripheral cytokines and immune cells to enter the brain, further exacerbating neuroinflammation.[16] Furthermore, chronic stress, a major risk factor for depression, activates SRC kinases in brain regions like the hippocampus and prefrontal cortex (PFC).[17] Finally, SRC kinases may influence glucocorticoid receptor signaling, which is often impaired in MDD, contributing to hypothalamic-pituitary-adrenal (HPA) axis dysregulation.[18] It should be noted that SRC inhibitors (e.g., dasatinib, saracatinib) are being explored for their potential to modulate neuroinflammation and synaptic plasticity.[19,20] Therefore, targeting SRC kinases could offer a novel approach to treating depression, especially in inflammation-driven

cases. However, no study has examined the role of SRC family kinases.[21]

Nano-selenium is a nanoscale form of selenium with enhanced bioavailability and absorption.[22] A selenium-containing compound known as 3-[(4-chlorophenyl)selanyl]-1-methyl-1H-indole (CMI) has been shown to alleviate depression-like behaviors and cognitive impairments in animal models by modulating oxidative stress and inflammatory pathways.[23] In mouse models subjected to stress, CMI effectively reversed stress-induced depressive behaviors by reducing levels of corticosterone (a stress hormone), enhancing antioxidant activities, and inhibiting oxidative stress.[24] Additionally, nano-selenium has been also linked to improvements in neuro-inflammation and BBB integrity, both critical factors in maintaining mental health and combating mood disorders.[25] Research suggests that nano-selenium can enhance cognitive functions by reducing oxidative damage and inflammation in the brain. Nano-selenium may help improve overall cognitive performance, particularly in conditions associated with chronic stress or neurodegenerative diseases.[26] Recent animal study has suggested that nano-selenium can influence JAK/STAT signaling pathway involved in MDD. However, its specific effects on c-SRC gene expression and the implications for MDD pathophysiology remain unexplored.[27] The current study aims to address this knowledge gap by investigating the impact of nano-selenium supplementation on c-SRC gene expression in patients with MDD. By focusing on c-SRC, this study seeks to provide novel insights into the molecular mechanisms underlying the antidepressant and neuroprotective effects of nano-selenium. We hypothesized that nano-selenium supplementation would decrease c-SRC gene expression compared to placebo, given its potential role in modulating intracellular signaling pathways involved in neuronal function and mood regulation.

Materials and Methods

Sampling: A triple-blind, parallel-group, randomized, placebo-controlled clinical trial was conducted to assess the effects of nano-selenium supplementation on c-SRC gene expression in patients with MDD. Participants were recruited from the Psychosomatic Clinic at Imam Khomeini Hospital Complex, Tehran, Iran, between December 2023 and July 2024. MDD was diagnosed by a neuropsychologist based on Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) criteria and the Structured Clinical Interview for DSM Disorders (SCID).[28,29] We enrolled participants based on the following criteria:

- Age: 18-50 years
- Diagnosis: Newly diagnosed with MDD based on DSM-V criteria and the SCID
- Treatment history: No antidepressant use for at least three months prior to the study
- Body mass index (BMI): Between 18.5 kg/m² and 30 kg/m²
- Consent: Provided written informed consent to participate

Non-inclusion criteria:

- Medical conditions: History of autoimmune, neurological, thyroid, cancer, kidney, liver, cardiovascular, or respiratory diseases
- Psychiatric conditions: Other major psychiatric disorders besides MDD
- Lifestyle and dietary factors: Disordered eating habits or following a special diet
- High suicide risk
- Pregnant or breastfeeding women
- Use of nutritional supplements within six weeks before enrollment
- Alcohol, opioid, or cigarette use
- Participation in another clinical trial within the past two months

Study withdrawal criteria:

- Significant dietary or lifestyle changes
- Alteration in sertraline regimen (50 mg/day)
- Development of inflammatory diseases lasting more than one week
- Worsening depressive symptoms requiring additional treatment
- Suicidal ideation reported during the study.

Using block randomization, participants received either nano-selenium (55 µg/day, n = 25) or the placebo (corn starch placebo, n = 25) for 12 weeks, with both groups also receiving sertraline (50 mg/day) throughout the study. Allocation concealment was ensured using sealed, opaque, sequentially numbered envelopes. Compliance was monitored through weekly phone and text check-ins. Participants attended two clinic visits – at baseline and at 12-week follow-up – where adherence was assessed by capsule count and self-reports. High adherence was defined as consuming at least 80% of the provided capsules. Participants were instructed to maintain consistent dietary and physical activity habits throughout the trial to minimize bias.

Participants' height was measured using a

wall-mounted tape measure to the nearest 0.5 cm while subjects were without shoes. The weight (in kilograms) and BMI (kg/m^2) was recorded at baseline and end of the study with barefoot and light clothing and standing using InBody H30N (Korea).

At the initiation, participants completed a general demographic questionnaire. Dietary selenium intake was evaluated using three 24-hour dietary recalls at baseline and final and then calculated using Nutritionist IV software, adapted for Iranian foods.

Measurement of c-SRC gene expression: Peripheral blood samples (3 ml) were collected from participants at baseline and after 12 weeks, between 8:00-10:00 AM. The samples were processed at the Hematology Department of Iran University of Medical Sciences, Tehran.

Ribonucleic acid (RNA) extraction: RNA was extracted from white blood cells (WBCs) using NorexPlus RNA extraction kits (NAT Biotech, Iran). RNA purity was assessed using a NanoDrop spectrophotometer, and only samples with an A260/A280 absorbance ratio of 1.8-2.2 were considered acceptable.

Complementary deoxyribonucleic acid (cDNA) synthesis: cDNA was synthesized using 500 ng of extracted RNA with the NATScript cDNA synthesis kit (NAT Biotech, Iran). The cDNA samples were stored at -20 °C until real-time polymerase chain reaction (PCR) analysis.

Primer design and selection: Primers for c-SRC gene expression were selected based on previous studies and validated using Oligo7 software, Ensembl database, and Primer-BLAST (NCBI) to ensure specificity. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene for normalization.

Reverse transcription quantitative PCR (RT-qPCR) analysis: Gene expression levels were assessed using real-time RT-qPCR with SYBR Green Master Mix. Amplifications were performed in a real-time PCR system (Rotorgene Corbett) under optimized conditions.

Gene expression calculation: The ΔΔCt method ($2^{\wedge -\Delta\Delta Ct}$) was applied to determine relative c-SRC gene expression changes, with GAPDH as the reference gene.

Nano-selenium and placebo capsule preparation

Nano-selenium synthesis: Nano-selenium was synthesized by dissolving 221.8 mg of selenium dioxide in 250 ml of deionized water and stirring at 500 rpm. A solution of 1524.6 mg of ascorbic acid in 50 ml of deionized water was added dropwise, leading to the formation of 121 mg of nano-selenium. The solution was stirred for 2 hours, followed by the addition of 50 mg of low-molecular-weight chitosan (LMWC), dissolved in 10 ml of 10% acetic acid, to coat the nanoparticles. The final chitosan-coated nano-selenium was collected by centrifugation and characterized using scanning electron microscopy (SEM), energy dispersive X-ray (EDAX), and dynamic light scattering (DLS) analysis.

Nano-selenium capsule preparation: To obtain a solid formulation, the synthesized nanoparticles were mixed with 400 g of corn starch, dried at 30 °C for 24 hours, and further processed to a final weight of 1100 g. The powder was ground, sieved, and encapsulated into 2200 capsules (500 mg each), with each capsule containing 55 µg of nano-selenium.

Placebo capsule preparation: Placebo capsules were prepared using pre-sieved corn starch powder, with 500 mg per capsule, yielding 2200 placebo capsules. Both nano-selenium and placebo capsules were produced using a semi-automatic capsule filling machine to ensure consistency.

Randomization was performed using block randomization via randomization.com. Allocation concealment was ensured using sealed, opaque, sequentially numbered envelopes. The study was triple-blind: participants, interviewers, and data analysts were blinded. The manufacturer coded the supplements to ensure blinding. Participants were randomly assigned in a 1:1 ratio to the nano-selenium group or the placebo group.

Sample size was based on a previous study examining selenium's impact on patients with type 2 diabetes.[30] With a significance level of 0.05 and 90% power, a minimum of 21 participants per group was required. To account for dropouts, each group included 25 participants. Data analysis was performed using SPSS software (version 24, IBM Corporation, Armonk, NY, USA). Normality was tested with skewness, histograms, box plots, quantile-quantile plots (Q-Q plots), and the Shapiro-Wilk test. Normally-distributed data were reported as mean ± standard deviation (SD), and non-normal data as median [interquartile range (IQR)]. For comparing two groups, we used independent t-tests for normally-distributed variables, Mann-Whitney tests for non-normally-distributed variables, and chi-square tests for categorical variables. For within-group comparisons, paired t-tests were used for

normally-distributed variables, and Wilcoxon tests for non-normally-distributed variables. Between-group comparisons were conducted using analysis of covariance (ANCOVA), adjusting for baseline c-SRC expression. This information is included in the statistical analysis subsection with significance set at $P < 0.05$.

Ethical approval: The research adhered to the principles of the Declaration of Helsinki and was approved by the Research Ethics Committees of Iran University of Medical Sciences (Approval ID: IR.IUMS.REC.1402.206, dated 2023-06-13). It was registered with the Iranian Registry of Clinical Trials (IRCT) (registration number: IRCT20091114002709N62, dated 2023-07-29). Written informed consent was obtained from all participants, and the study followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Results

Of 171 individuals screened between December 2023 and July 2024, 50 met the criteria and joined the study. From the placebo group, four participants were excluded (one due to not taking at least 80% of the capsules, one due to unwillingness to continue the study, one due to pregnancy, and one due to anxiety symptoms). From the nano-selenium group, four participants were excluded (two due to not taking the capsules in the prescribed amount, and two due to personal preference). Eight participants (four from each group) were excluded, leaving 21 in each group for the final analysis, with an 84% retention rate in both groups. No adverse events or safety concerns were reported by participants during the study.

Per-protocol analysis was conducted in the remaining participants (Figure 1). BMI was measured at baseline and final, and no significant differences were found between the nano-selenium and placebo groups at baseline (25 ± 2 vs. 25 ± 3 kg/m², respectively; P = 0.791) and final (25 ± 2 vs. 25 ± 3; P = 0.931). Baseline and final dietary selenium intake was assessed, and no significant differences were observed between the nano-selenium and placebo groups at study initiation (1.2 ± 0.8 vs. 0.9 ± 1.0 micrograms, respectively; P = 0.276) and final (1.1 ± 0.8 vs. 1.1 ± 1.0, respectively; P = 0.754).

Due to the potential for differential responses to nano-selenium based on disease severity, baseline details for both the nano-selenium and placebo groups separated by MDD severity are provided in table 1. In the placebo group, patients with moderate depression were older (35.00 ± 8.69 years) and had higher BMI (27.09 ± 2.57 kg/m²), while in the nano-selenium group, patients with severe and very severe depression were older (38.57 ± 5.13 years) and had higher BMI (25.02 ± 3.13 kg/m²). No significant differences were observed in baseline characteristics between groups.

Initial Mann-Whitney test showed no differences in the expression levels of c-SRC genes between the nano-selenium and placebo groups [median (Q1, Q3): 0.0032 (0.0014, 0.0067) vs. 0.0037 (0.0013, 0.0151), respectively; P = 0.831].

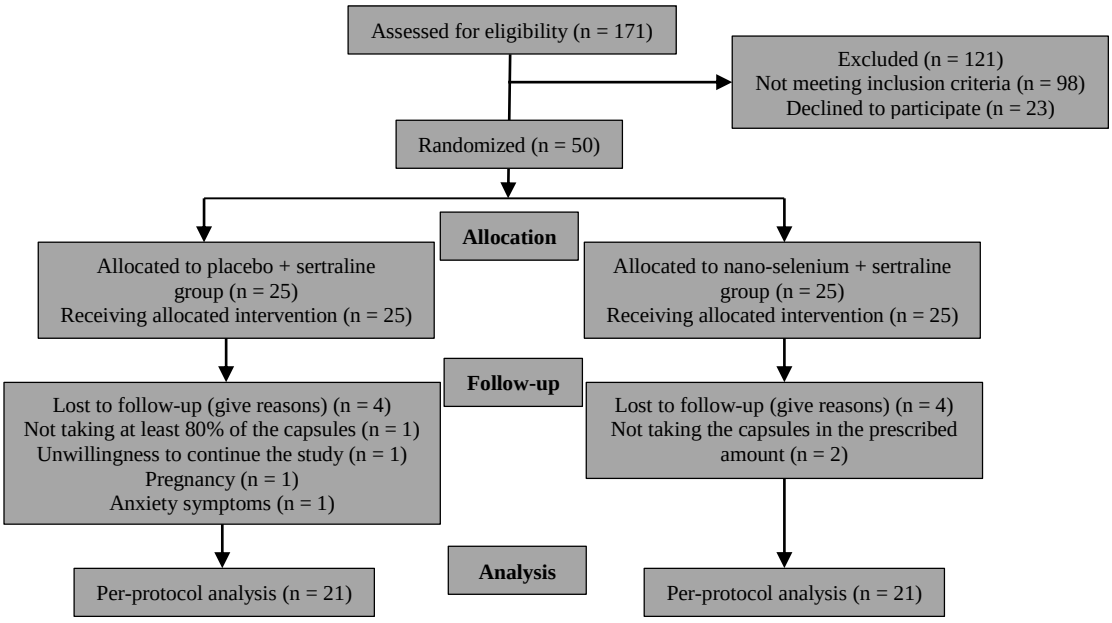

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

Table 1. General demographic data for participants with major depressive disorder (MDD) receiving nano-selenium + sertraline or placebo + sertraline separated by disease severity

Variable	Placebo + sertraline group			P*	Nano-selenium + sertraline group			P*
	Patients with mild depression (n = 8)	Patients with moderate depression (n = 5)	Patients with severe/very severe depression (n = 8)		Patients with mild depression (n = 8)	Patients with moderate depression (n = 6)	Patients with severe/very severe depression (n = 7)	
Age (year) (mean ± SD)	32.75 ± 3.20	35.00 ± 8.69	30.75 ± 5.23	0.426	32.50 ± 7.46	36.00 ± 8.34	38.57 ± 5.13	0.272
BMI before (kg/m^2) (mean ± SD)	25.15 ± 2.88	27.09 ± 2.57	22.67 ± 3.65	0.064	24.68 ± 2.51	24.45 ± 1.60	25.02 ± 3.13	0.918
Sex (women) [n (%)]	6 (75.0)	5 (100)	8 (100)	0.314	8 (100)	6 (100)	6 (85.7)	0.619
Employed (no) [n (%)]	4 (50.0)	2 (40.0)	0 (0)	0.133	4 (50.0)	3 (50.0)	3 (42.9)	0.735
Married (yes) [n (%)]	3 (37.5)	4 (80.0)	1 (12.5)	0.056	4 (50.0)	4 (66.7)	6 (85.7)	0.376

*Using one-way analysis of variance (ANOVA), chi-square, or Fisher's exact test as appropriate

BMI: Body mass index; SD: Standard deviation

After the intervention, c-SRC expression levels significantly decreased in the nano-selenium and placebo groups [median change (Q1, Q3): -0.0031 (-0.0065, -0.0005) vs. -0.0021 (-0.0085, 0), respectively; P < 0.05], though no differences were found between the groups (P = 0.606) (Table 2). Fold change analysis indicated a decrease in c-SRC expression in the placebo group, contrasting with a slight increase in the nano-selenium group; however, these changes were not statistically significant (P = 0.778). The average fold change values for the placebo versus intervention groups were as follows: c-SRC: 0.98 ± 1.78 vs. 1.19 ± 2.82 as shown in figure 2.

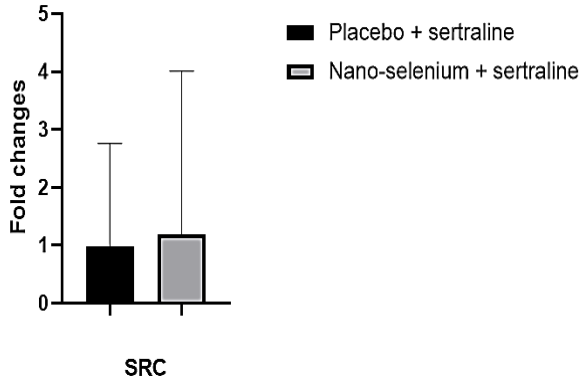

Figure 2. The average fold change values for the placebo versus intervention groups

Discussion

The current study sought to examine the effects of nano-selenium supplementation on c-SRC gene expression in patients with MDD receiving sertraline treatment. Despite the hypothesized benefits of nano-selenium as an adjunct therapy based on its antioxidant properties, our findings did not reveal any statistical and clinical significant differences in the c-SRC gene expression between the nano-selenium plus sertraline group and the placebo plus sertraline group.

Several possible explanations could account for the lack of significant effects observed in this trial. First, it is plausible that the SRC signaling pathway is not critically involved in the antidepressant effects mediated by nano-selenium in the context of MDD. While preclinical animal study has suggested a potential role of the JAK/STAT signaling pathway in MDD,[27] the translation of these findings to clinical populations remains complex and SRC may not be incorporated in this effect.

Additionally, differences in baseline genetic variability and genetic polymorphisms among participants in the SRC gene or its pathway components might have contributed to variability in the response to nano-selenium.[31] Such polymorphisms may influence SRC gene expression or function, potentially affecting the responsiveness to interventions like nano-selenium.[31] Given the role of SRC kinases in intracellular signaling pathways, it is plausible that polymorphisms affecting SRC expression or activity could influence the efficacy of interventions targeting these pathways.[31] Therefore, the lack of significant findings in our study might be due to underlying genetic variations among participants that modulate SRC gene expression or function, thereby diminishing the potential impact of nano-selenium supplementation. It is possible that the primers employed in our study were not optimized to detect all transcript variants or polymorphic regions of the SRC gene. In cases where participants harbor genetic variants in the primer-binding sites, gene expression levels might not have been accurately quantified, leading to potential underestimation of SRC expression in certain individuals.[32]

Another potential factor is the concurrent use of sertraline, kind of SSRIs, which could have masked the effects of nano-selenium. Since we included participants with various severity of depression in this study, we did not exclude participants from standard treatment with sertraline due to ethical considerations.

Table 2. The relative expression of SRC gene separated by nano-selenium supplementation and placebo groups ($2^{-(ct\ SRC-\ ct\ GAPDH)}$)

Group	Placebo group	Nano-selenium group	P*	P***
	Median (Q1, Q3)	Median (Q1, Q3)		
SRC before	0.0037 (0.0013, 0.0151)	0.0032 (0.0014, 0.0067)	0.831	
SRC after	0.0010 (0.0003, 0.0023)	0.0004 (0.0001, 0.0020)	0.159	0.325
Change	-0.0021 (-0.0085, 0.0000)	-0.0031 (-0.0065, -0.0005)	0.606	
P**	0.012	0.005		

*Using the Mann-Whitney test; **Using the Wilcoxon test; ***Using the analysis of covariance (ANCOVA) adjusted based on baseline SRC expression

SSRIs exert broad effects on neurotransmission, inflammation, and oxidative stress pathways, and it is conceivable that these mechanisms overlap or overshadow the specific effects of nano-selenium on the SRC pathway. This interaction warrants further investigation in studies employing different antidepressant classes or treatment-naïve populations.[33,34] Sertraline has been shown to influence immune signaling pathways, including modulating inflammatory cytokines like IL-6 and TNF-α, which are known to interact with the JAK/STAT pathway and potentially with SRC kinases.[35] Since SRC kinases are involved in intracellular signaling that can be influenced by inflammation and serotonin signaling, sertraline's effect on inflammatory responses might alter SRC expression directly or indirectly, making it difficult to discern the isolated effect of nano-selenium.[35-37] Sertraline also affects the serotonergic system, which can influence neuroplasticity and neuroinflammation, processes that may interact with the SRC pathway in the central nervous system (CNS).[35,38] SSRIs like sertraline may modulate the expression of genes involved in neuroplasticity and neuroinflammation, which includes potential effects on SRC gene expression.[38,39] Sertraline has been shown to influence various signaling molecules such as brain-derived neurotrophic factor (BDNF), glycogen synthase kinase-3β (GSK-3β), and others involved in cellular signaling, which might interact with the SRC pathway in the brain.[40]

The heterogeneity of MDD as a clinical entity may also contribute to the observed null findings. MDD is a multifactorial disorder with diverse underlying pathophysiological mechanisms, and it is unlikely that a single pathway or biomarker can fully account for its complexity.[41]

It is also worth noting that the lack of significant differences in SRC pathway-related gene expression does not preclude the possibility of other beneficial effects of nano-selenium in MDD. Nano-selenium may influence other molecular pathways, such as JAK/STAT signaling or indoleamine 2,3-dioxygenase 1 (IDO1) expression, which were not the primary focus of this study but have been implicated in the pathophysiology of depression.[27] Future research with broader molecular profiling could provide a more comprehensive understanding of the mechanisms underlying the effects of nano-selenium.

The dose and duration of nano-selenium supplementation may have been insufficient to elicit measurable changes in SRC pathway-related gene expression. Although the selected dose was based on the recommended dietary allowance (RDA) for demonstrating its safety and potential efficacy,[42] it is possible that a higher dose or longer duration of supplementation is required to produce significant effects in patients with MDD. Future studies could explore dose-response relationships to determine the optimal supplementation regimen.[43]

Additionally, the use of peripheral blood samples as a proxy for CNS activity poses inherent limitations. While peripheral biomarkers offer practical advantages, they may not fully reflect molecular changes occurring in the brain; therefore, collecting cerebrospinal fluid (CSF) may better reflect the effect.[44] We collected peripheral blood samples due to its being minimally invasive, whereas CSF collection requires a lumbar puncture, which is painful, costly, and carries risks (e.g., infections, headaches).[45] Besides, ethical concerns make it difficult to obtain CSF samples from patients with non-severe MDD in routine clinical studies.[46] In comparison, peripheral blood sampling allows for larger participant recruitment compared to CSF-based studies. Peripheral blood sampling is easier and cheaper to conduct longitudinal studies with multiple blood collections over time.[46] However, while peripheral blood cells can reflect some systemic immune processes, they may not accurately capture SRC expression in neurons or glial cells, where it plays a key role in neurotransmission and synaptic plasticity. SRC expression varies across different cell types [e.g., neurons, astrocytes, microglia vs. peripheral blood mononuclear cells (PBMCs)] and SRC activation in brain tissue may not be reflected in peripheral blood, leading to potential false-negative results in peripheral-based studies.[47] Although we aimed to control confounding effects like lifestyle, peripheral blood gene expression is influenced by diet, stress, medications, circadian rhythms, and immune status, which may introduce variability in results. Unlike CSF, which is more directly linked to CNS physiology, blood-based markers may be affected by external factors.[48]

Based on our knowledge, we examined the effect of nano-selenium supplementation on MDD for the first time. Nano-selenium has been shown to have higher absorption rates from the intestinal lumen compared to traditional selenium supplements. This improved bioavailability means

that smaller doses can achieve similar or greater biological effects. The nanoscale form allows for a controlled release of selenium, which helps maintain effective levels in the body without reaching toxic thresholds. This characteristic is particularly beneficial in therapeutic applications, where precise dosing is crucial. Nano-selenium retains strong antioxidant capabilities, which can mitigate oxidative stress without contributing to pro-oxidant effects that can occur with high doses of inorganic selenium. This balance is essential for reducing the risk of adverse effects associated with oxidative damage.[49] While nano-selenium is generally safer, some potential side effects still exist. Although less common, gastrointestinal issues can occur if nano-selenium is consumed in excess. However, these side effects are typically less severe than those associated with inorganic forms of selenium.[19] We did not observe any side effects in the current study.

Another strength of this study was the randomized, parallel, triple-blind clinical trial design. None of the participants, the project managers, and the analysts were aware of whether the subjects were assigned to the nano-selenium or placebo group.

Limitations: However, this study had some limitations. Although the 12-week supplementation period was selected based on previous studies,[50] 12 weeks seems to be insufficient to investigate the effects of nano-selenium supplementation on gene expression. Moreover, although the selenium dose was selected based on the RDA, it is recommended that future studies test different doses up to the upper limit.

Conclusion

Our findings suggest that nano-selenium supplementation does not significantly modulate SRC pathway-related gene expression in patients with MDD receiving sertraline. These results highlight the need for further research to elucidate the role of nano-selenium and its potential mechanisms of action in depression. Future studies should consider exploring alternative molecular pathways, optimizing supplementation protocols, and identifying subgroups of patients who may derive greater benefit from this intervention. It is suggested for future studies that participants be stratified based on sertraline treatment or treatment-naïve status to better understand how sertraline may modify the effect of nano-selenium. Future studies could incorporate genetic screening to identify SRC polymorphisms among participants. This approach would allow subgroup analyses to determine whether specific genetic variants are associated with differential responses to nano-selenium supplementation, thereby providing a more nuanced understanding of its therapeutic potential in MDD. Besides, it is suggested for future studies that peripheral blood and functional imaging be combined. If CSF collection is not feasible, functional imaging techniques [e.g., positron emission tomography (PET) scans for neuroinflammation] could help validate peripheral findings. Future studies should assess whether SRC-related changes are more evident in high-inflammation subgroups.

Conflict of Interests

The authors declare no conflict of interest in this study.

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