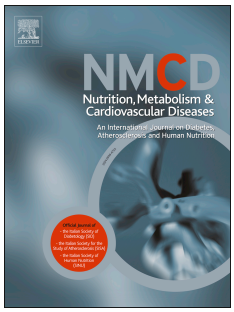

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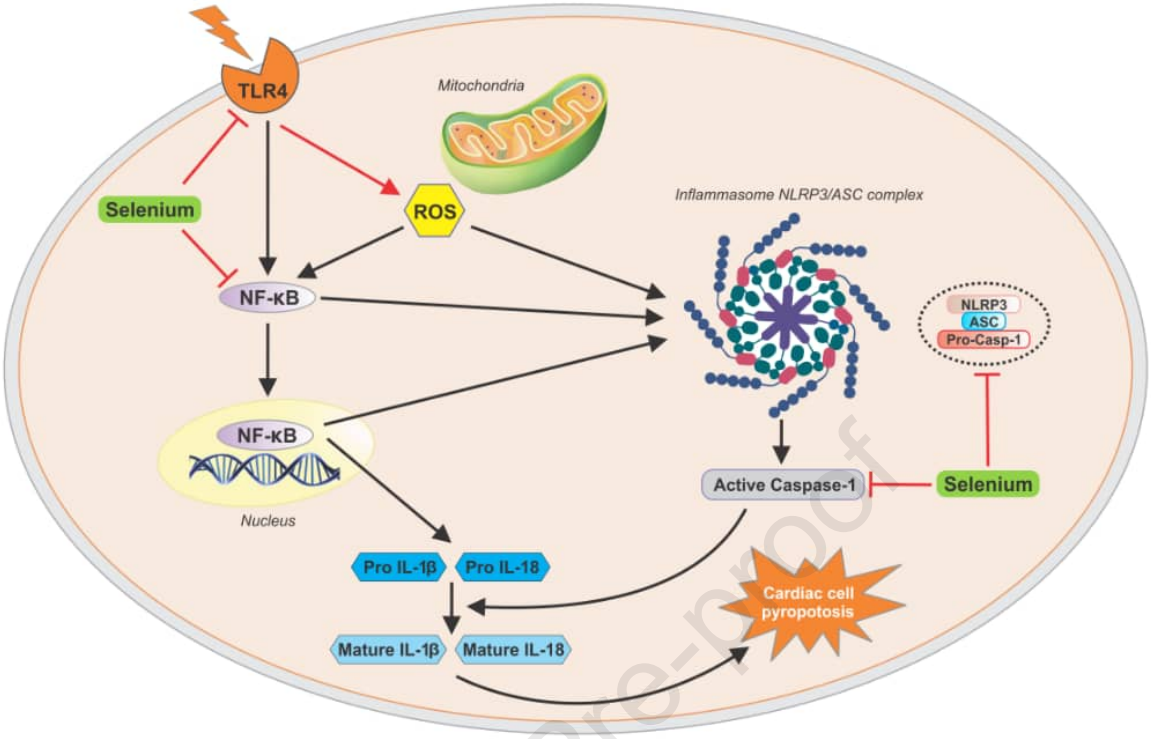

Sodium selenite and Se-enriched yeast supplementation in atherosclerotic patients: Effects on the expression of pyroptosis-related genes and oxidative stress status

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Abstract

Background and Aims

Atherosclerosis is a chronic inflammatory disorder of the arterial wall is the main leading cause of the cardiovascular disease (CVD). Caspase-dependent pyroptosis plays a pivotal role in the pathogenesis of CVD. Selenium (Se) is an important component of the antioxidant defense plays a crucial role in cardiovascular health. This study aimed to investigate the effects of daily consumption of sodium selenite and Se-enriched yeast on the expression of pyroptosis-related genes, and biomarkers of oxidative stress in patients with atherosclerosis.

Methods and Results

In this randomized, double-blinded, placebo-controlled clinical trial involving 60 patients with atherosclerosis were recruited. Participants received 200 µg/day of sodium selenite, Se-enriched yeast, or placebo for 8 following weeks. The pyroptosis-related genes' mRNA expression in peripheral blood mononuclear cells (PBMCs) was assessed before and after the intervention. Also, the levels of superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO), and glutathione peroxidases (GPX) were measured at baseline and following the intervention. Following sodium selenite and Se-enriched yeast supplementation, the relative expression levels of TLR4, ASC, NLRP3, and NF-κB1 were significantly

downregulated ($p<0.05$). Furthermore, the changes in GPX were significantly increased after selenite and yeast supplementation ($p<0.05$). Also, selenite and yeast consumption caused a statistical significant decrease in the change of MDA level ($p<0.05$).

Conclusion

In summary, findings showed that Se supplementation may reduce inflammation through down-regulation of some pro-inflammatory genes and improving antioxidant defenses in atherosclerosis patients. Further research is required to come to a definite conclusion of selenium supplementation on the CVD risk.

This study was registered on the Iranian Registry of Clinical Trials website (identifier: RCT20110123005670N28; https://www.irct.ir/).

Keywords: Cardiovascular disease; Atherosclerosis; Inflammation; Pyroptosis; Selenium; Yeast

Introduction

Coronary artery disease (CAD) and its major complication, myocardial infarction, were the focus of research for decades. CAD, which is characterized by inflammation within the atherosclerotic coronary vessel wall, has high mortality and morbidity worldwide [1, 2].

Based on pioneering evidence, inflammation plays a pivotal role in the initiation and progression of atherosclerotic plaque [3]. Low-grade systemic inflammation (meta-inflammation) is well known as an independent cardiovascular risk factor that contributes significantly to the atherogenic process and oxidative stress [4]. Pyroptosis which is a pro-inflammatory caspase-1 mediated programmed cell death has been widely shown in various cell types, including endothelial cells and cardiomyocytes [5, 6] accompanied by marked endothelial cell injury and producing various pro-inflammatory cytokines including interleukin (IL)-1β and IL-18 [7, 8].

Cell death is visible in the atherogenic process and has a vital role in the development and progression of atherosclerotic lesions. Pyroptosis contributes to the formation and progression of the atherogenic process through the release of inflammatory factors. NLRP3 is the most important inflammasome which is the link between lipid metabolism and inflammation since, cholesterol crystals, and oxidized low-

density lipoprotein (oxLDL) can activate the NLRP3 inflammasome to induce pyroptosis[9, 10].

New emerging evidence confirms that pyroptosis plays a major role in CVDs. Various triggers such as high-fat/high-sugar cause mitochondrial dysfunction. Moreover, ROS overproduction increased the nuclear translocation of NF-κB and activated NLRP3 inflammasome inducing pyroptosis occur extensively in ECs, CMs, and VSMCs[11, 12].

Various kinds of inflammasomes, including NLRP3 (chiefly), NLRP1, NLRP6, NLRP9, NLRC4, Pyrin13, and AIM2 can trigger this caspase-1 mediated cell death pathway. NLRP3 as an important NOD-like receptor protein can recognize danger signals and initiate an inflammatory response [13]. Furthermore, the activation of the NLRP3 inflammasome- (IL)-1β pathway is conceived to promote atherosclerosis [14]. NLRP3 engages apoptosis-associated speck-like protein (ASC) and leads to the activation of caspase-1. Subsequently, the activated caspase-1 can cleave the pro-IL-1β and pro-IL-18 molecules and leads to the onset of a widespread inflammatory cascade. Toll-like receptors (TLRs)/ nuclear factor kappa-light-chain-enhancer of activated *B* cells (NF-κB) signaling pathway is one of the upstream triggers of the NLRP3 inflammasome/ (IL)-1β pathway [15]. Recent research has revealed a close link between inflammation and redox balance. The absence of antioxidant defense

proteins (e.g., superoxide dismutases (SOD), nitric oxide (NO), and glutathione peroxidases (GPX)) can facilitate the activation of NLRP3 inflammasome cascade and be considered as a major trigger for cardiovascular disease (CVD) [16].

Epidemiologic data support an intensive association between circulating some nutrients levels with cardiovascular risk. Selenium (Se) as a part of GPX plays an important role in antioxidant mechanisms in the human body [17]. The relationship between blood Se levels and risk of CAD has been investigated in pioneering studies revealing a decreased concentration of total Se level in blood can be one of the risk factors for heart disease [18-20]. Recently, Seas an effective radical-tapping antioxidant has been extensively investigated pharmacologically for modulation of inflammation.

Selenium is an essential trace element that plays a pivotal role in the enzymatic antioxidant defense system. It is a component of numerous antioxidant enzymes including GPxs and SOD, protects cells against damage and mitigate oxidative stress, detoxifies peroxides such as Malondialdehyde which is the product of lipid peroxidation. Thus it is involved in the antioxidant mechanisms that prevent oxidant damage[21, 22].

In this study; we assessed the effect of sodium selenite and enriched yeast with sodium selenite supplementation on the expression level of pyroptosis-related genes

in peripheral blood mononuclear cells (PBMCs) in patients with atherosclerosis. Furthermore, the level of serum SOD, malondialdehyde (MDA), NO, and GPX as prognostic markers of oxidative stress were measured.

Material and methods

Participants

From July to October 2019, a total of 60 patients with atherosclerosis in coronary arteries who were referred to Shahid Madani heart Hospital of Tabriz University of Medical Sciences, Tabriz, Iran, were recruited in this study. Two cardiologists diagnosed atherosclerosis through careful examinations and angiography. The inclusion criteria required subjects to have atherosclerosis diagnosed with angiography with more than 50% stenosis at least in one artery, aged 18–80 years, and body mass index (BMI) between 25–35 kg/ m^2. The exclusion criteria were as follows: having malignant tumors, chronic liver or kidney autoimmune disorders, pregnancy or breastfeeding, infectious diseases, taking any medicine that would influence lipid and glucose metabolism, antioxidants and dietary supplements over 6 months before the study, and patients who followed less than 90% of their intervention. The study was conducted following the Declaration of Helsinki and the protocol of the study was approved by the Human Research Ethics Committee of Tabriz University of Medical Sciences (Ethical code: IR.TBZMED.REC.1398.1187. All of the participants completed written informed consent at baseline. The study

protocol was registered at the Iranian Registry of Clinical Trials (IRCT ID: IRCT20110123005670N28).

Study design

Participants were randomly allocated to one of three groups (Se-enriched yeast (n = 20), sodium selenite (n = 20), and placebo group (n = 20)) in a parallel design (allocation ratio 1:1) using the mean (±standard deviation [SD]) of GPX of Ying Hu et al., study [23] Based on a confidence interval (CI) of 95% and power of 80% and considering a possible 10% dropout rate. A randomized block procedure was used to divide participants into one of three treatment blocks, using a computer-generated allocation schedule. In this randomized, double-blind, placebo-controlled clinical trial, participants, the trial investigators, and the local physician who allocated the patients to the study groups were blinded. Sodium selenite and Se-enriched yeast capsules were synthesized at Nutrition Research Center, Tabriz University of Medical Sciences, Iran. Se-enriched yeast is produced by growing Saccharomyces cerevisiae in Si-rich media, and its preparation and optimization of culture conditions are reported in detail earlier in the Suhajda study [24]. According to the previous study and tolerable upper levels of Se, the Se-enriched yeast and sodium selenite intervention dose were considered 200 µg/day [25].

Patients in the yeast group received one capsule of 200 µg Se-enriched yeast daily, and the selenite group received a 200 µg/day capsule of sodium selenite daily before

meal for 8 following weeks. The placebo group was given the same amount of starch capsules over the same period. The capsules were similar in shape, size, and color. Yeast, selenite, and placebo capsules were provided to groups weekly. Adherence to the supplements was assessed by counting the remaining capsules returned by the patients. Adherence was defined as the consumption of ≥ 90% of the supplements.

Selenium preparation

As preparation of Se-enriched yeast required Selenium to be in the soluble form, the alkali metal selenite was produced from metallic Selenium through the selenous acid formation procedure. According to this method, Selenium powder was gradually dissolved in hot boiling nitric acid until a clear solution of selenous acid formed in reaction with an alkali metal base which forms alkali metal selenite. The boiling was continued until the elimination of excess amount of nitric acid. The so prepared selenous acid was reacted with NaOH as an alkali metal base with a stoichiometric ratio. The prepared crystals were separated from the selenite sodium salt. To identify of selenite phase, XRD characterization was applied.

Se-enriched yeast preparation

The preparation of Se-enriched yeast was carried out by bioaccumulation of Selenium under appropriate conditions. As reported in different investigations, the drab yeast color after bioaccumulation shows that Selenium is mainly organic. By knowing this fact, the optimum amount of Selenium in the culture medium was

adjusted. The culture medium was prepared using molasses and adding essential elements that improve the growth of yeast. As Selenium present in the culture medium has an inhibitory effect on yeast growth, the selenite sodium salt addition at the optimum amount was conducted at the logarithmic phase. At the end of the process, the amount of Selenium in the prepared yeast was measured by inductively coupled plasma mass spectrometry (ICP-MS). The value of Selenium in the capsules was adjusted according to this amount.

Isolation of peripheral blood mononuclear cells (PBMCs)

Five ml of venous blood samples were collected from all patients to vacuum collection tubes containing EDTA (Vacutainer K2E) before and after the intervention. All blood samples were obtained in the fasting state at 8 am in the same conditions at baseline and at the end of the intervention. All genes' expression were evaluated using RT-qPCR assay from all samples which randomized across the 3 mentioned arms.

PBMCs were separated from buffy coats by Ficoll-Histopaque solution gradient (ficollpaque, GmbH) and washed once in sterile phosphate-buffered saline (PBS) before RNA isolation.

RNA isolation protocol

Using Ambion Trizol LS reagent (Thermo Fisher Scientific, USA), high-quality total RNA was isolated from PBMCs according to the manufacturer's instructions. RNA quality and quantity were confirmed by the relative absorbance ratio at A260/280 and A260/230 using a spectrophotometer (NanoDrop™ One/OneC Microvolume UV, Thermo Scientific). Using the total isolated RNA, random hexamer primer, and reverse transcriptase according to the manufacturer's protocol (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit, USA), total RNA was reverse transcribed to complementary DNA (cDNA). According to the manufacturer's protocol, cDNA was prepared from 1 μg of mRNA and diluted to a final concentration of 50 ng/μl. The prepared cDNA was stored in -20 ºc for further analyses. Roche Life Science Thermal Cycler (LightCycler® Instrument, Germany) made running polymerase chain reaction (PCR).

Real-time PCR

Real-time reverse transcription PCR (qRT-PCR) was performed to measure ASC, TLR4, NF-κB1, Caspase-1, NLRP3, IL-1β, and IL-18 genes' expression at the mRNA level using the Roche Life Science/real-time PCR. SYBR Green Master mix (AMPLIQON, Denmark) was used for quantitative real-time PCR (qPCR). The amplification program consisted of 1 cycle at 95 °C for 5 min, followed by 40 cycles with a denaturing phase at 95 °C for 10 s, annealing phase of 35 s at 54 °C, and an extension phase of 20 s at 72 °C. The sense and antisense primers sequences were

designed using PrimerBank, NCBI, and summarized in Table 1. The mRNA was normalized against the β-actin mRNA as the reference gene, and the relative mRNA abundance was calculated using the $2^{(-\Delta\Delta CT)}$ method [26]. All samples were run in triplicate.

Assessment of serum SOD, MDA, NO, and GPX

Five ml of blood samples were obtained in the fasting state at 8 am in the same conditions before and after the intervention. The samples were centrifuged at 2500 rpm for 10 min, and then the serum samples were stored at -80°C for further experiments. The levels of SOD, MDA, NO, and GPx were measured using platinum enzyme-linked immune sorbent assay (ELISA) kits (Navand Salamat, Iran).

Statistical analysis

SPSS statistical software (SPSS Inc., Chicago, IL, USA, version 23) was used to analyze the data. One-sample Kolmogorov- Smirnov test was used to assess the normality of variables. Logarithmic transformation was performed for skewed data. Data are presented as the mean (SD) and mean (SEM). A One-way ANOVA test was used to evaluation of the differences in fallowed by Post-hoc analysis for two-by-two group comparisons. The changes in SOD, MDA, NO, and GPx were determined with the following formula:

[(After intervention values - Baseline values)].

For data analysis, the amounts of the mRNA were normalized to the β-actin transcript level as an internal reference, using $\Delta\Delta$CT comparative method. Then, fold change was calculated using the $2^{-\Delta\Delta CT}$ equation, as the relative expression of post intervention/placebo.

$$\text{Fold change (FC)} = \frac{(\text{Efficiency}_{\text{target}})^{\Delta CT \text{ target (control - sample)}}}{(\text{Efficiency}_{\text{reference}})^{\Delta CT \text{ ref (control - sample)}}}$$

. P-value <0.05 was considered statistically significant. Figures were created with Prism software, version 6.0 (GraphPad, CA, USA).

Results

The flowchart of the study is presented in Figure 1. A total of 49 patients completed the study. Three patients in the placebo group, four from the yeast group, and four from the selenite group were drop out for reasons independent of the interventions. According to the participant's reports, supplementation with Se-enriched yeast and sodium selenite did not have any side effects or symptoms. Table 1 presents the demographic characteristics of the study population. According to the One-way ANOVA analysis, there were no significant differences in terms of baseline, general and demographic properties between the three studied groups (P>0.05).

Effects of selenite and Se-enriched yeast supplementation on pro-inflammatory signaling pathway in atherosclerotic patients

Fig 2 shows the influence of selenite and Se-enriched yeast supplementation on the TLR signaling pathway.

The expression level of TLR-4 was significantly lower in selenite group (fold change: 0.51± 0.16, p<0.001) and yeast group (fold change: 0.84± 0.12, p= 0.005) compared to the placebo group (fold change: 1.40± 0.11) (Fig2A). Regarding caspase-1 mRNA expression, there were no significant differences between study groups (P>0.05) (Fig2 B).

A significant reduction was detected in ASC expression in selenite (fold change: 0.55± 0.23, p<0.001) and yeast group (fold change: 0.92± 0.16, p<0.001) relative to placebo group (fold change: 2.55± 0.37) (Fig2C). The mRNA expression levels of NLRP-3 were shown in Fig 2 D. The expression levels in the selenite (fold change: 0.57±0.11, p=0.006) and yeast (fold change: 0.74±0.16, p=0.013) group were significantly down-regulated relative to the placebo (fold change: 1.53±0.15).

In line with NLRP-3 findings, NF-κB1 expression levels were decreased significantly in selenite (fold change: 0.10±0.03, p<0.001) and yeast (fold change: 0.18±0.06, p<0.001) group in comparison with placebo (fold change: 1.80±0.29) (fig 3 A)

Regarding IL-1β and IL-18 mRNA expression, there was no significant difference between the studied groups (P>0.05) (Fig 3B, C).

Effects of selenite and Se-enriched yeast supplementation on oxidative stress status in atherosclerotic patients

To investigate the effect of selenite and Se-enriched yeast supplementation on indices of oxidative stress, the serum levels of GPX, SOD, NO, and MDA were measured using ELISA kits. The baseline measures of these factors were not significantly different between studied groups (data are not shown) ($P>0.05$).

As observed in fig4 A, changes in GPX were significantly increased after selenite ($p<0.001$) and yeast ($p=0.001$) supplementation in comparison with placebo. However, the changes of SOD and NO were not remarkably different between the studied groups ($P>0.05$) (Fig4B, D). Furthermore, the mean difference of MDA was significantly declined in the selenite ($p=0.049$) and yeast ($p=0.004$) group in comparison with the placebo group (Fig5C).

Discussion

This clinical trial investigated the effects of Se supplementation (selenite and Se-enriched yeast) on pyroptosis pathway-related genes' expression and oxidative stress status in atherosclerotic patients for the first time.

Genes' expression data showed significant down-regulation of pro-inflammatory genes including TLR4, NLRP3, ASC, and NF-kB in both supplementation groups compared to placebo. Interestingly, selenite reduced the genes expression more than Se-enriched yeast. Additionally, we found an elevated serum level of GPX in

selenite and Se-enriched yeast groups compared to placebo. Selenite increased GPX level more efficiently than yeast. Moreover, the mean change of MDA was significantly declined in the selenite and Se-enriched yeast groups, compared to placebo.

Inflammation and oxidative stress are related to the pathogenesis of CVDs and atherosclerosis [27]. Seleno-proteins exert their cardio-protection role by regulating redox balance in the body. The indirect antioxidant potential of Selenium arises from its incorporation into the structure of seleno-proteins such as GPX [28, 29]. Moreover, Se decreases inflammatory markers and regulates oxidative stress by reducing ROS and inhibiting the NF-kB signaling pathway [30]. Seleno-proteins inhibit the binding of NF-kB to DNA, thereby reducing inflammation and regulating vasoconstriction and coagulation [31, 32]. Additionally, Selenoenzymes play a crucial role in the cellular defense against oxidative stress by decreasing free radicals and the regeneration of other antioxidants [33].

Previous studies have demonstrated Selenium's antioxidant potential and immunomodulatory role via regulating antioxidative enzymes activity and the production of cytokines [34-36]. In line with our results, Liu et al. [37] showed that Se-enriched yeast decreased the expression levels of TLR-4 and NF-κB and regulated oxidative stress balance by enhancing the activity of serum GPX and total antioxidant capacity (T-AOC) and reducing MDA in piglets. In addition, various

animal studies have demonstrated that Se increased the activity of antioxidant enzymes such as GPX and regulated pro-inflammatory cascade by down-regulation of NLRP3, caspase-1, and IL-1β [38-42].

Moreover, a study on atherosclerotic patients showed a negative correlation between serum levels of Se and concentration of pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α [43]. Beyond all that was said, Selenium may exert its anti-inflammatory effects by suppressing TLR4 and its downstream signaling pathway [44, 45]. TLR4, as a crucial factor in the mediation of inflammatory pathways, can lead to the activation of NF-kB and play critical roles in the induction of pro-inflammatory genes expression, including IL-1β.

It has been reported that Selenium deficiency is directly associated with heart failure, and conversely, administration of Se in patients with a low level of blood Se could significantly improve the CAD status [29, 46]. Furthermore, several animal studies have shown increased inflammation in Selenium-deficient animals and attenuated it by administration of Selenium supplementation [47].

A study on the Selenium-deficient porcine aorta model showed that Selenium deficiency significantly increased the gene expression of NLRP3 inflammasome and its downstream molecules, including ASC, caspase-1, IL-18, and IL-1β [48].

Interestingly, various researches and clinical trials suggest that Selenium supplementation may benefit subjects with low Selenium status at the baseline. In a

randomized controlled trial conducted on chronic heart failure (CHF) patients, Raygan et al. found that Se-enriched yeast supplementation for 12 weeks has potential effects in alleviating inflammatory status by reducing high sensitivity C-reactive protein (hs-CRP) and increasing total antioxidant capacity and glutathione levels without any impact on NO and MDA concentration [49]. Interestingly, some previous studies have revealed that seleno-methionine or Se-enriched yeast cannot increase selenoprotein activity in subjects with already sufficient amounts of Selenium [50].

Conclusions

Although yeast is a suitable carrier for selenium biotransformation, our study results showed more efficient effects for sodium selenite in reducing the pyroptosis-related genes' expression and GPX level than yeast. Previous research suggested that sodium selenite may be more biologically available for GPX activity than the Se-enriched yeast. Based on the evidence, when Se-enriched yeast was consumed in high doses, the serum GPX activity reached a plateau at approximately 0.1 ppm Se [51] and this can be a justification for the results observed in our study.

Generally, our findings showed that Se supplementation may reduce inflammation through down-regulation of some pro-inflammatory genes and improving antioxidant defenses in atherosclerosis patients. To the best of our knowledge, this study is the first study that reports the protective potency of Selenium

supplementation against CAD via the pyroptosis inflammatory pathway. However, the underlying mechanisms require more detailed research. This study highlights the critical role of Selenium in the prevention and management of CAD.

Limitations

This study is the first investigation regarding the molecular inflammatory signaling pathways following selenite and Se-enriched yeast supplementation in patients with atherosclerosis. The present study had a few limitations. The genetic differences of participants' short duration of intervention and limited sample size were the main limiting factor in our research. Also, we did not assess the baseline level of Selenium in subjects. Furthermore, the variability in data may be due to differences in study design, dosage, and duration of supplementation. Further detailed investigations are needed to generalize the results of this investigation to the general population and other CVDs.

Competing Interests

The authors declare no conflicts of interest.

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Authorship

ATE and NR designed the study with contributions from SG. NR drafted the manuscript with contributions from NMA, SB, and ANS. MN, MKK, SA, and HS were lab investigators in the study. NR, EM, and MTS contributed to data collection or analyses. SG, NR, and ATE reviewed and approved the final version of the paper and are the guarantor of this work.

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Figure legends:

Fig1) Study flow diagram.

Fig2) Fold change of TLR-4, caspase-1, ASC and NLRP-3 mRNAs expression in three studied groups after interventions. Quantification was done by real-time PCR. The bar graphs presented the mean of fold change ± SEM. Data analysis was done using paired T-test for comparisons between mean baselines and after intervention values (*P < 0.05 vs. baseline) and ANOVA for analyzing the differences among group means. Tukey was selected as a posthoc test (**P < 0.05 vs. Placebo). P < 0.05 statistically significant. TLR-4: Toll-like receptor4, ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain, NLR-3: Nod-like receptor 3.

Fig3) Fold change of NF-κB1, IL-1β and IL-18 mRNAs expression in three studied groups after interventions. Quantification was done by real-time PCR. The bar graphs presented the mean of fold change ± SEM. Data analysis was done using paired T-test for comparisons between mean baselines and after intervention values (*P < 0.05 vs. baseline) and ANOVA for analyzing the differences among group means. Tukey was selected as a posthoc test (**P < 0.05 vs. Placebo). P < 0.05 statistically significant. NF-κB1: Nuclear factor-κB, IL: Interleukin.

Fig 4) Mean change of GPXS, SOD, MDA, and NO in three studied groups after interventions The bar graphs presented the mean of changes ± SEM. Data analysis was done using ANOVA Tukey was selected

as a posthoc test. (*P < 0.05, **P<0.01, ***P<0.001).GPS: Glutathione peroxidases, SOD: Superoxide dismutase, MDA: Malondialdehyde, NO: Nitric oxide

Graphical abstract) Schematics of the proposed pathway for inhibitory connection between Selenium and pyroptosis cell death. Danger signals in Atherosclerotic patients detected by Toll-like receptors (TLRs). This initiates a signaling cascade that leads to cell death by pyroptosis or caspase-1-dependent cell death (through activation of nuclear factor-κB (NF-κB), stimulation of inflammasomes, and production of inflammatory cytokines including IL-1β and IL-18). Selenium can modulate the immune response and alleviate pyroptosis cell death.

→ : Increased level: Inhibition

Variables		groups			P-value*
		Selenium-enriched Yeast (n= 16)	Sodium Selenite (n= 16)	Placebo (n= 17)	
Age (Year)		59.06± 8.55	58.62± 9.68	53.58± 13.75	0.287
Weight(kg)		81.03± 11.35	81.18± 9.43	83.71± 13.73	0.759
Height(m)		1.68± 0.07	1.70± 0.05	1.70± 0.08	0.868
BMI(kg/m^2)		28.56± 3.63	27.86±2.21	28.29± 3.07	0.774
SBP(mm Hg)		110.62±15.37	111.87±15.90	119.70±13.04	0.096
DBP (mm Hg)		70.90±8.98	76.25±7.41	75.29±10.22	0.251
Gender	Male	12 (75)	16 (100)	15 (88.2)	0.097**
	Female	4 (25)	0 (0)	2 (11.8)	

Table 1) Baseline characteristics of the study participants

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. Qualitative variables are reported as frequency (percent). Quantitative variables are reported as mean ± SD. *P-value is reported based on the Kruskal-Wallis test; ** P-value is reported based on the Chi-Square test. $p<0.05$ is considered statistically significant.

Table 2. Sequences of Genes' primers for qRT-PCR

Gene	Primers
NF-κB1	Forward CTGTCCTTTCTCATCCCATCTT
	Reverse TCCTCTTTCTGCACCTTGTC
NLRP3	Forward AGCATCGGGTGTTGTTGTCA
	Reverse AAGATAGCGGGAATGATGATATGAG
Caspase-1	Forward CCTGGTGTGGTGTGGTTTA
	Reverse ATCCTTCTCTATGTGGGCTTTC
TLR2	Forward GCAGAAAACAACCTGAACC
	Reverse GCTTGTTCCTCACTACTCTC
TLR4	Forward AGCCATGATCAGCCTCACAC
	Reverse GGGATTTGGGAAGGTGCAGA
IL-18	Forward GACTGTAGAGATAATGCACCC
	Reverse TTTCTCACACTTCACAGAGAT
IL-1β	Forward CTCTCACCTCTCCTACTCACTT
	Reverse TCAGAATGTGGGAGCGAATG
ASC	Forward GAGCCCACCAACCCAAGCA
	Reverse GTCCTTGCAGGTCCAGTTCCA
β-actin	Forward GGTGAAGGTGACAGCAGT
	Reverse TGGGGTGGCTTTTAGGAT

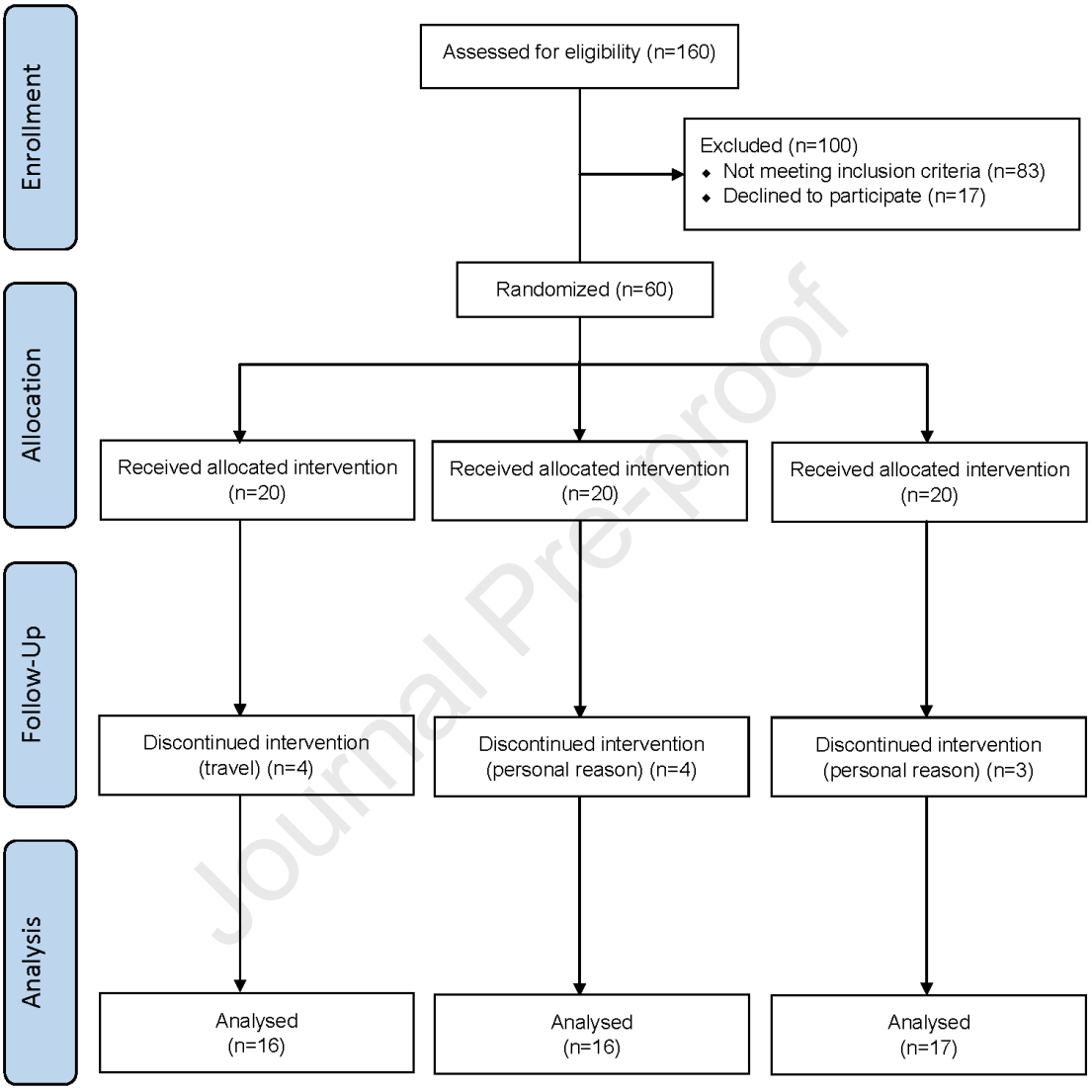
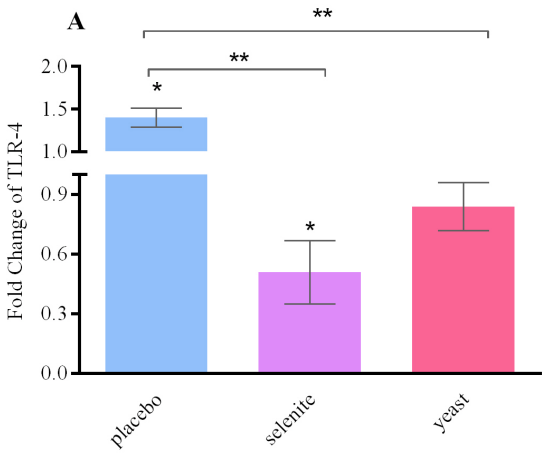
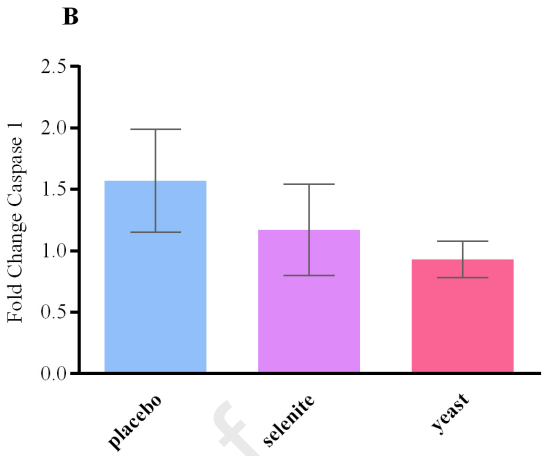
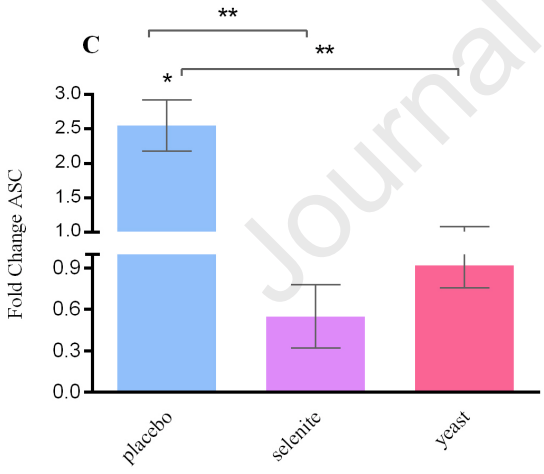
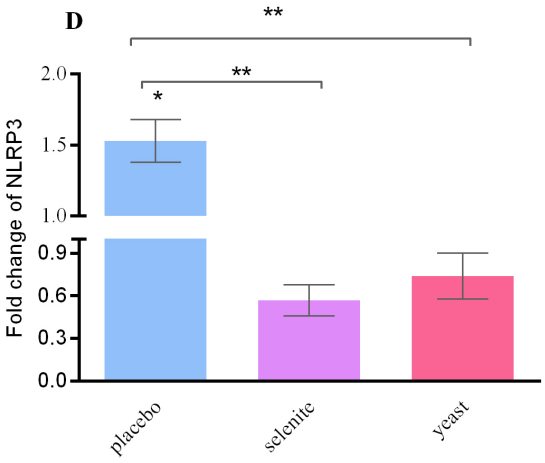


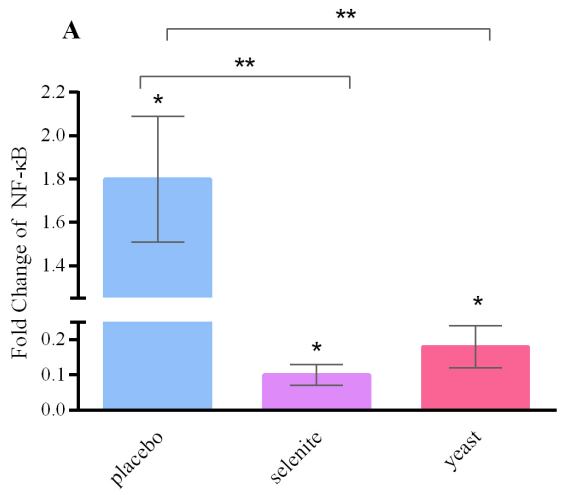
Fig. 1. Study flow diagram.

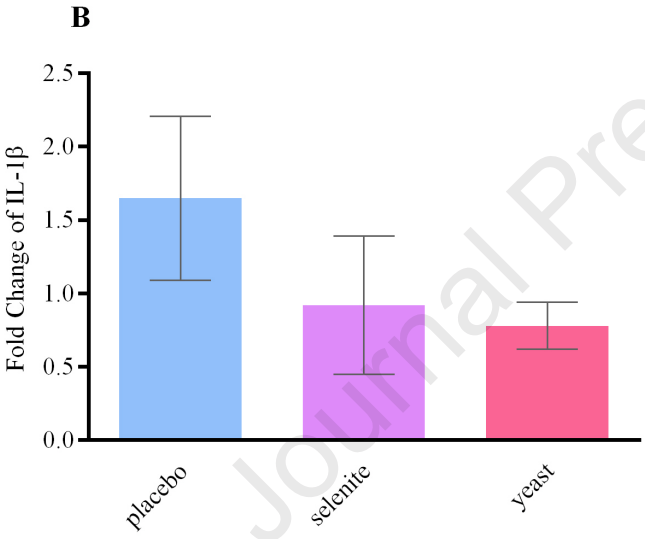


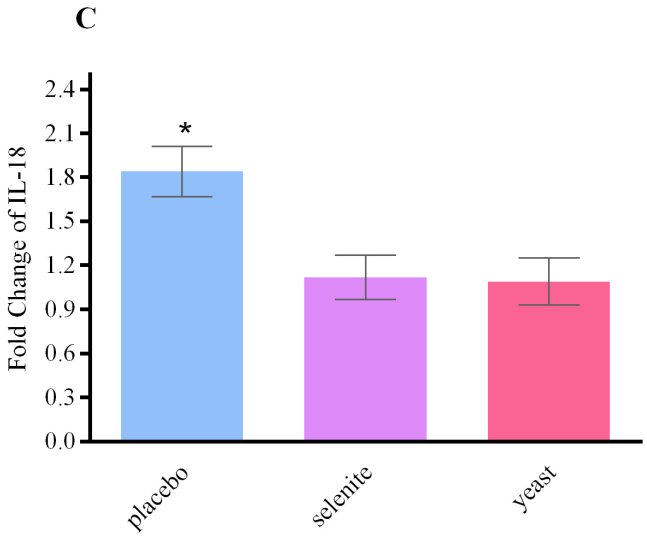


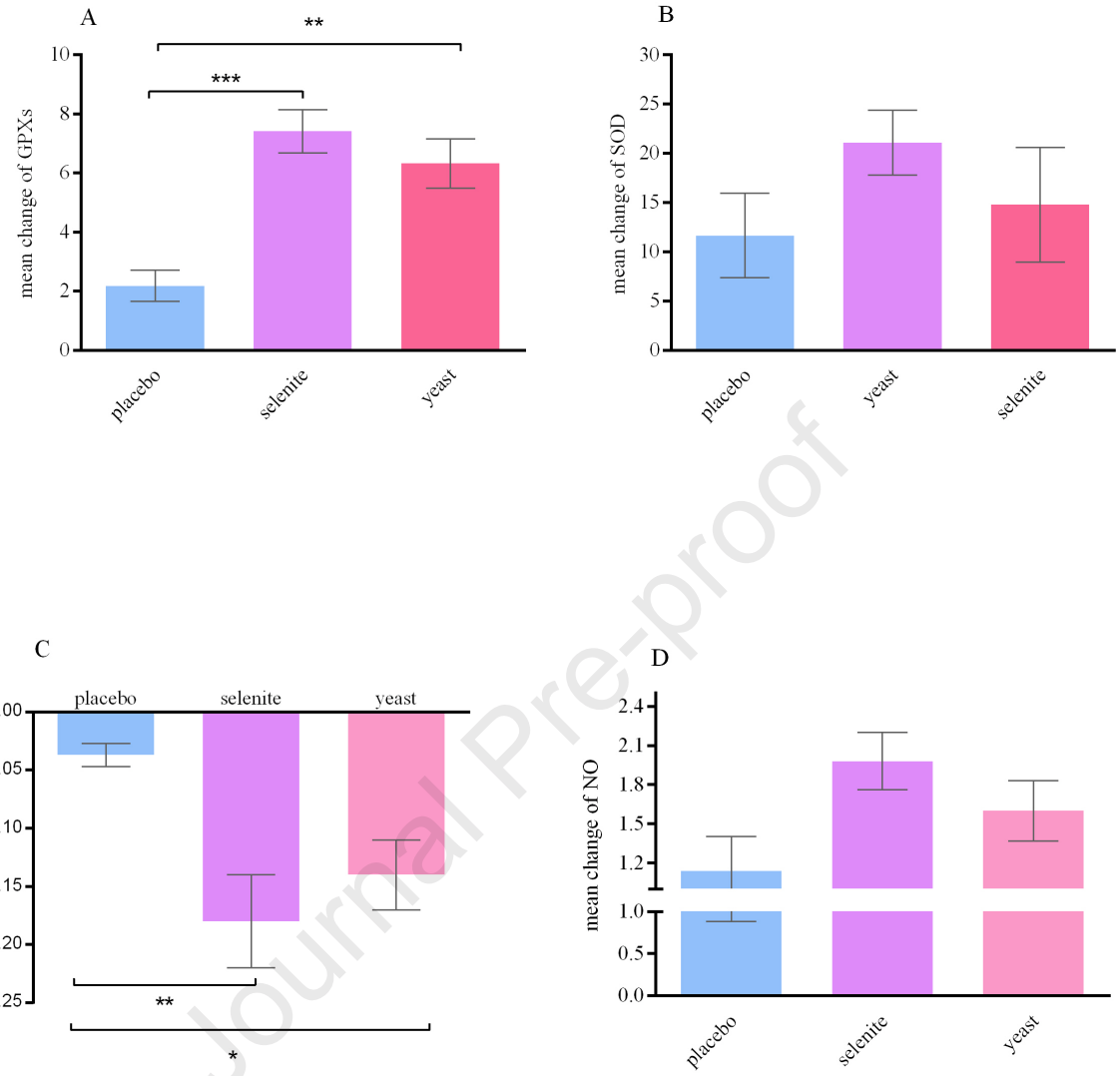












Highlights

- Cardiovascular disease prevalence has been rising more rapidly worldwide
- Low-grade systemic inflammation is well known as an independent cardiovascular risk factor
- Pyroptosis is characterized by release of pro-inflammatory cytokines including IL-1β and IL-18
- Selenium may exert beneficial actions for attenuating inflammation

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Declarations of interest: none

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