RESEARCH ARTICLE

The Effect of Selegiline on Reducing Oxidative Stress in Polymorphonuclear Blood Cells (Lymphocytes) Isolated from Patients with Rheumatoid Arthritis

Purbajit Chetia[1,2], Abdul B Ahmed[3*]

[1]*Assam Science and Technology University, Jalukbari, Guwahati, Assam, India.*
[2]*Department of Pharmacology, NETES Institute of Pharmaceutical Science, NEMCARE Group of Institute, Mirza, Guwahati, India.*
[3]*Girijananda Chowdhury Institute of Pharmaceutical Science, Girijananda Chowdhury University, Tezpur, Sonitpur, Assam, India.*

Received: 14th January, 2023; Revised: 28th March, 2023; Accepted: 9th July, 2023; Available Online: 25th September, 2023

ABSTRACT

Rheumatoid arthritis (RA) is a persistent condition resulting in an inflammatory response abide by the pathological mechanism of autoimmunity that causes the cartilage, bone, and joint tissues to deteriorate, lowering one's quality of life in general. Several studies have suggested a significant association between oxidative stress caused by leukocyte-mediated inflammatory responses and the conversion of a substantial amount of oxygen into diverse free radicals, resulting in oxidative harm, including the chronic nature of rheumatoid arthritis. The utilization of DMARDs which are known as biologic and conventional disease-modifying antirheumatic drugs, has exhibited encouraging results. However, it is crucial to prioritize the discovery of novel and efficacious medications for rheumatoid arthritis in order to address the constraints associated with current therapeutic approaches. Selegiline, a synthetic drug primarily employed to manage Parkinson's disease, predominantly interferes with the MAO-B enzyme, also known as monoamine oxidase. Additionally, it has been demonstrated to possess free radical-neutralizing properties. The goal of this study is to assess the effectiveness of selegiline in reducing oxidative stress indicators in peripheral blood mononuclear lymphocyte cells isolated from individuals diagnosed with RA. Peripheral blood was collected from 20 RA patients in accordance with the American College of Rheumatology standard. Lymphocytes were isolated following incubation with 1.5 µg/mL phorbol myristate acetate (PMA) and treatment with different concentrations (50–200 µg/mL) of selegiline. This study reveals that selegiline in concentrations of 150 and 200 µg/mL was effective enough to alleviate oxidative stress by scavenging free radicals and improving the level of natural defensive enzymes playing as antioxidants. Therefore, it can be concluded that the MAO-B inhibitor selegiline has potential as a non-toxic repurposed medicine for curtailing the pathological effects of oxidative overload during RA, unlocking the opportunity for further investigation into the impact on other inflammatory cells, such as neutrophils.

Keywords: Selegiline, Inflammation, Blood lymphocytes, Oxidative stress, Rheumatoid arthritis.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.3.06

How to cite this article: Chetia P, Ahmed AB. The Effect of Selegiline on Reducing Oxidative Stress in Polymorphonuclear Blood Cells (Lymphocytes) Isolated from Patients with Rheumatoid Arthritis. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):494-500.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic illness with 19th and 20th century roots. Being a chronic autoimmune disease, it destroys cartilage, bones, and joint tissues and lowers the quality of life.[1,2] Contemporary research has documented a destructive inflammatory process within the body, as evidenced by the presence of synovial cellular infiltration and peripheral blood immune cells such as monocytes, lymphocytes, and neutrophils.[3] Polymorphonuclear neutrophils, including lymphocytes, are essential donors to synovial inflammation and subsequent joint injury.[4] Similarly, mesenchymal cell-derived cells, notably fibroblast-like synoviocytes (FLS), mediate direct tissue damage and perpetuate the complicated disease process by altering themselves morphologically and phenotypically in autoimmune joint diseases like RA.[5,6] Furthermore, several studies have proposed a notable involvement of oxidative stress induced by leukocyte-mediated inflammatory responses, involving the conversion

**Author for Correspondence: baquee1977@gmail.com*

of a considerable quantity of oxygen into various free radicals, resulting in oxidative damage and the chronicity of rheumatoid arthritis.[7,8] Reactive oxygen species (ROS) and mitochondrial damage are closely associated with quite a few significant pathological processes in rheumatoid arthritis (RA). Consequently, the modulation of mitochondrial function and mitigation of oxidative overload have established themselves as prominent therapeutic targets for treating RA.[9,10] Indeed, it has been observed that ROS has the tendency to regulate the activity and the processes associated with the activation of immune cells.[11] Certain evidence postulates an increase in ROS and RNS within the circulatory system of rheumatoid arthritis patients compared to that of a normal individual, accompanied by a fading of the endogenous enzymatic and non-enzymatic defense mechanisms.[12] The task at hand involves identifying a unique therapeutic intervention for RA, which is complicated by a poor understanding of the underlying molecular processes and the role of autoantibodies.[13] Despite recent progress in therapeutic strategies, this difficulty persists. While contemporary pharmaceutical interventions, such as biologic and synthetic disease-modifying antirheumatic drugs (DMARDs), NSAIDs, and glucocorticoids, have demonstrated promising outcomes, the need for early diagnosis and prompt evaluation cannot be overstated in achieving optimal treatment effectiveness.[14,15] However, apart from the negative consequences, a significant subset of patients, referred to as "difficult to treat" or "refractory RA", persist with symptoms regardless of medical intervention with two or more DMARDs.[16,17] The development of new effective drugs for rheumatoid arthritis is imperative to rectify existing therapy's limitations, enhance patient outcomes, implement more personalized approaches, and ultimately advance the management of this chronic condition. Selegiline is now utilized in the management of Parkinson's disease, principally exerting its therapeutic effects by permanently inhibiting the "B" isoform of the enzyme monoamine oxidase (MAO).[18] This inhibition serves to impede the metabolism and degradation of dopamine, resulting in enhanced potency and a more favorable pharmacological profile.[19] Numerous significant studies have documented the neuroprotective, antioxidant, anxiolytic, and depressive properties of selegiline in various organs, which are not solely attributed to its MAO-B inhibitory effects but rather arise from its modulation of cellular oxidation mechanisms and mitochondrial enzymes.[20,21] Therefore, considering the aforementioned evidence, it is reasonable to suggest that selegiline may be beneficial while managing RA through the suppression of oxidative damage and inflammatory biomarkers induced by free radicals. Therefore, this investigation focuses on analyzing the potential preventative mechanism of selegiline in inhibiting oxidative stress in isolated polymorphonuclear blood lymphocyte cells of individuals diagnosed with RA.

MATERIALS AND METHOD

Sample Population

The Institutional Ethics Committee approves this study protocol with no. IEC/2021/270-A. This study included 20 patients with RA employed from the outpatient unit of NEMCARE Hospital, Guwahati, Assam, India, and Govt. Ayurvedic College, Guwahati, Assam, India, according to the American College of Rheumatology criteria.[24] with informed consent, excluding those with viral polyarthritis, psoriatic arthritis, systemic lupus erythematosus, scleroderma, hepatic, or renal impairment. Steroid-using patients are also precluded from this study (Table 1).

Peripheral Blood Mononuclear Cells Isolation from RA Blood and Treatment with Selegiline

The blood from healthy individuals and RA patients was directly collected in heparin-containing vials. As per the guidelines provided by the manufacturer (Hisep^{TM} LSM 1073), PBML was separated after centrifugation at a force of 400 g for 30 minutes. Due to the differential migration of cells as per density gradient, numerous layers were formed where PBML was found to be in the banded plasma interphase. Formed pellets mostly contain erythrocytes. Following the collection of plasma, including thrombocytes, lymphocytes were isolated and washed with phosphate buffered saline.[25] Cells were quantified and trypan blue exclusion staining confirmed cell viability.[26] PBML cells (1×10^6 per mL) isolated from the RA patients were pretreated with 1.5 µg/mL phorbol myristate acetate (PMA) for one hour. In addition, the cells were subjected to various doses (ranging from 50 to 200 µg/mL) of selegiline in Krebs-Henseleit solution for a duration of 2 hours. Subsequently, the cells were lysed in 2 mL of phosphate buffer (0.02M, pH 7.4) containing 0.25% sodium dodecyl sulfate (SDS) through freeze-thawing at -20°C for three times. After estimating the protein concentration following standard protocol,[27] cell lysate was employed to conduct additional oxidative biomarker tests.

Superoxide Anion Estimation in PBML

The superoxide anion (O^{2-}) assessment in isolated lymphocyte cells was conducted by performing the nitro blue tetrazolium assay based on its capacity to form purple formazan formazan.[28,29] Briefly, lymphocyte cells at a quantity of 1×10^6/mL were subjected to treatment with Krebs-Henseleit buffer accompanied by 1-mg/mL of nitroblue tetrazolium (NBT) for a duration of 45 minutes. The cells were lysed using a phosphate buffer solution (80 mM, pH 7.8) supplemented with 0.045% gelatin including 5% SDS. Following centrifugation for 5 minutes at 3000 rpm, the collected supernatants' absorbance was measured at 540 nm for formazan development and 450 nm for protein existance. Finally, the presence of superoxide anion was

Table. 1: Brief of study population

RA Patient's Data (N=20)	
Age	*30–70 Years*
Gender (Male: Female)	13:7
Disease Duration	1–30 Years
ACR Criteria	Yes
DMARD Received	Yes
Antioxidants Received	No

estimated through the following formula: F_{540} = (Absorbance at 540 nm – Absorbance at 450 nm)/0.49.[25]

Nitrite Estimation in PBML

Nitrite level was measured through the Griess assay for the purpose of determining the presence of nitric oxide (NO) in PBML with minute alteration.[30] One part of cell lysate is added to nine parts of 5% TCA, followed by centrifugation for 5 minutes at 8000 rpm. Absorbance at 546 nm was recorded after the incubation of the supernatant blended with an equivalent quantity of Griess reagent. The sodium nitrite standard curve was utilized to figure out the nitrite concentration.[31]

Superoxide Dismutase Activity in PBML

Depending on the protocol mentioned in Kakkar *et al.*, 1984.[32] Superoxide dismutase (SOD) activity was evaluated. Briefly cell lysate (200 µL), phenazine methosulfate (PMES, 186 mM), and nicotinamide adenine dinucleotide (NADH, 780 mM) are blended together with a subsequent addition of nitro blue tetrazolium (NBT, 300 mM), followed by incubation for 5 minutes at 30°C in an unilluminated area. After adding glacial acetic acid (500 µL) to discontinue the reaction, absorbance is recorded at 560 nm. The concentration of enzyme needed to reduce chromogen production by 50% in a test is considered one unit of SOD activity.[33]

Lipid Peroxidation Estimation in PBML

The protocol described in Ohkawa *et al.*, 1979[34] was employed for lipid peroxidation estimation with minor modifications. After blending 1-mL of cell lysate with an equal volume of 10% TCA and allowing it to settle down for 30 minutes at room temperature, centrifugation was done (3000 g × 10 min). Further, 0.5 mL of 1% TBA was incorporated into the collected supernatant (1.5 mL), followed by water bath heating for about 45 minutes at 95°C, forming pink chromogen. Absorbance was observed at 532 and 600 nm, where malondialdehyde (MDA) concentration (mM) = (A532 - A600)/155.[35]

Reduced Glutathione Estimation in PBML

Following the potency of thiol (SH) group of glutathione (GSH) to reduce dithiobis-2-nitrobenzoic acid (Ellman's reagent), developing a yellow-colored compound, GSH content was estimated.[36] After blending 200 µL of cell lysate with an equal volume of 10% TCA and allowing it to settle down for 30 minutes at room temperature, centrifugation was done (3000 g × 10 min). 2.4 mL of EDTA (0.02M) was added to 200 µL of the collected supernatant and kept for 10 minutes in an ice bath, followed by centrifugation (3000 g × 15 min). Further Tris buffer (1-mL, 0.4 M), supernatant (1-mL), and 0.05 mL Ellman's Reagent (0.01M in methanol) were well mixed with the vortex. Absorbance was recorded at 412 nm within a time frame of approximately 2 to 3 minutes after the incorporation of Ellman's reagent. GSH was calculated with the help of a standard curve.

Statistical Analysis

The findings are reported in the format of the mean ± SEM for a sample size, N= 20. The SPSS software computed the statistical significance (*p-value*). A post hoc test (Tukey honestly significant difference, commonly referred as HSD) was used after conducting one-way analysis of variance (ANOVA). The significance levels were established at a threshold of *$p < 0.05$ and **$p < 0.01$, denoting statistical significance and strong statistical significance when comparing the experimental group with the matching control group.

RESULTS

The vitality of the cells was verified using trypan blue exclusion techniques. This technique is a crucial staining approach to specifically deceased cells (blue color), while uncolored pigmentation is observed in live cells with intact cellular membranes. A minimum viability threshold of 95% was deemed acceptable for subsequent inquiry.

The level of superoxide anion was estimated in the isolated peripheral blood mononuclear lymphocytes. The study observed a notable increase in the concentration of superoxide anions in the peripheral blood mononuclear lymphocytes (PBML) cells obtained from patients diagnosed with RA in contrast to healthy individuals. ($p < 0.01$). The increase in higher superoxide anions exhibits more oxidative overload in patients with RA.[37] Moreover, the samples were subjected to incubation with phorbol myristate acetate (PMS), resulting in an increased generation of superoxide anions. Selegiline at a concentration of 50 µg/mL did not have any remarkable effect on the decrease in superoxide production in the PMS-induced group. Nevertheless, in Figure 1, a notable impact was identified at a dosage of 100 µg/mL ($p < 0.05$). The extent of 150 µg/mL and 200 µg/mL of selegiline in curtailing the extent of Superoxide anion in the PMS-induced group was highly significant. ($p < 0.01$) Figure 2 indicates the capacity of selegiline reducing in NO production in PMA-induced isolated PBLM cells. After incubation with PMA, the increase level of NO production in PBML was highly significant. ($p < 0.01$). In addition, selegiline was also effective enough with high significance ($p < 0.01$) while preventing NO production in PMA-treated PBML cells in a way that depends on the concentration in a section of 100 to 200 µg/mL.

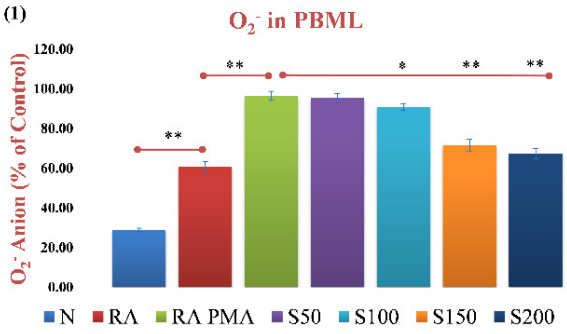

Figure 1: Superoxide anion assessment in PBML. N" represents normal human volunteers, "RA" represents isolated PBML of patients with Rheumatoid arthritis, "RA PMA" represents PMA treated RA and "S50, S100, S150, S200" represents PMA with Selegiline 50, 100, 150, 200 µg/mL treated RA

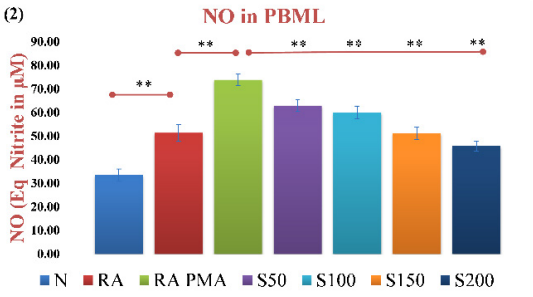

Figure 2: Nitric oxide assessment in PBML. "N" represents normal Human volunteers, "RA" represents isolated PBML of patients with Rheumatoid arthritis, "RA PMA" represents PMA treated RA and "S50, S100, S150, S200" represents PMA with selegiline 50, 100, 150, 200 µg/mL treated RA

Lipid peroxidation often occurs following oxidative overload as a consequence of free radicals giving rise to 4-hydroxynonenal (4-HNE) and MDA.[38] Polyunsaturated fatty acids (PUFAs) in phospholipids are the most abundant constituents within cellular membranes and are very sensitive to oxidation consequent to oxidative overload.[39] In this study, the malondialdehyde content was estimated and surprisingly increased ($p < 0.01$) in isolated PBML of patients with RA contrast to normal humans. After incorporation of PMA, the isolated PBML cells give rise to an elevated level of MDA content, perhaps due to more lipid peroxidation. ($p < 0.01$). Selegiline concentrations in a section of 50 to 200 µg/mL exhibit a concentration-dependent decrease in the MDA content in PBML (Figure 3). However, the effects of 50 and 100 µg/mL were deemed to be significant ($p < 0.05$) and 150 and 200 µg/mL appeared to be highly significant. ($p < 0.01$).

Reduced GSH in the body is one of the major oxidative biomarkers that continues to mitigate the oxidative overload represented by the increased emission of free radicals in RA patients.[40] Because of the potentiality of SOD in reducing oxidative stress by blocking excess free radicals like superoxide, it is regarded as an enzyme antioxidant in our bodies. Several lines of evidence have demonstrated a consistent diminution of GSH and SOD levels in persons diagnosed with RA. This decline can be attributed to the excessive consumption of these antioxidants by cells in order to alleviate the harmful outcomes

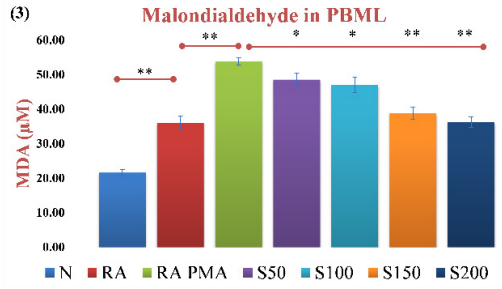

Figure 3: Malondialdehyde assessment in PBML. "N" represents normal Human volunteers, "RA" represents isolated PBML of patients with Rheumatoid arthritis, "RA PMA" represents PMA treated RA and "S50, S100, S150, S200" represents PMA with Selegiline 50, 100, 150, 200 µg/mL treated RA

of free radicals.[41] In our present evaluation also, both SOD ($p < 0.01$) and GSH ($p < 0.05$) appeared to be lesser in isolated PBML of persons with RA than those of normal individuals. A further decrease is observed after the treatment with PMA due to more production of free radicals. The SOD content in PBML increases with Selegiline concentration, with a span of 50 to 200 µg/mL (Figure 4). However, statistical analysis revealed that 50 and 100 µg/mL of selegiline had a significant influence ($p < 0.05$), but 150 and 200 µg/mL of concentrations had a higher degree of statistical significance ($p < 0.01$). Figure 5 depicts that 50 µg/mL of selegiline did not significantly impact the GSH increase in PBML. But at 150 and 200 µg/mL concentrations, selegiline's effectiveness in increasing the GSH level in isolated PBML is highly significant. ($p < 0.01$) while that of 100 µg/mL is significant. ($p < 0.05$).

DISCUSSION

A notable elevation in oxidative overload has been seen in those diagnosed with RA. The elevation in oxidative stress seen can be attributed to an upregulation in the formation of intracellular superoxide anions in PBML as well as in synovial fluid.[42] Pretreating PBML with phorbol myristate acetate (PMS) increases oxidative stress even more, as seen in the present study. On the other hand, an elevation in the generation of nitric oxide was also noted. Despite its role as a helpful chemical generated by nitric oxide synthase, nitric oxide exacerbates tissue degradation in rheumatoid arthritis by functioning as a proinflammatory mediator in the pathological

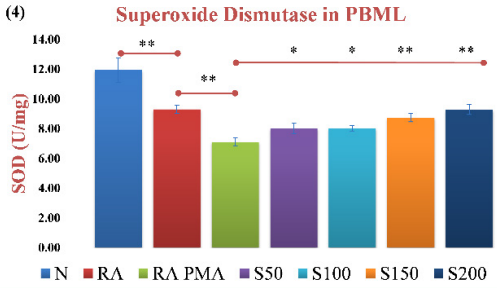

Figure 4: SOD assessment in PBML. "N" represents Normal Human Volunteers, "RA" represents isolated PBML of Patients with Rheumatoid Arthritis, "RA PMA" represents PMA treated RA and "S50, S100, S150, S200" represents PMA with Selegiline 50, 100, 150, 200 µg/mL treated RA

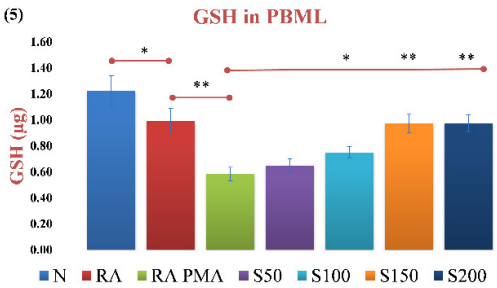

Figure 5: GSH assessment in PBML. "N" represents Normal Human Volunteers, "RA" represents isolated PBML of Patients with Rheumatoid Arthritis, "RA PMA" represents PMA treated RA and "S50, S100, S150, S200" represents PMA with Selegiline 50, 100, 150, 200 µg/mL treated RA

state.[43] Nitric oxide and superoxide anion are the main free radicals produced by lymphocytes in peripheral circulation.[44] It is noteworthy that the interaction of nitric oxide in a rapid manner along with superoxide anion results in the formation of peroxynitrite, hence exacerbating the pathological state.[45] The present study reveals that selegiline could minimize the level of superoxide anion and NO in the isolated PBML cells, mainly in 100, 150 and 200 µg/mL concentrations. The theoretical investigation of selegiline's free radical scavenging action has been previously conducted and demonstrated to be efficacious.[46] Selegiline possesses a propargylamine moiety that incorporates an acetylene group, enabling the donation of proton (H^+) ions for the purpose of scavenging free radicals.[47] Selegiline has also been shown to have *in-vitro* antiarthritic action in the past.[48] To our surprise, 150 and 200 µg/mL of selegiline provide excellent inhibition of lipid peroxidation in terms of curtailing the production of MDA in PBML. Reactive oxygen species (ROS) have the ability to initiate lipid peroxidation (LPO), which leads to the production of highly reactive MDA, causing functional and/or structural damage to the cell membrane. This mechanism entails the interaction of oxidizing agents with lipids that possess carbon-carbon double bonds, namely polyunsaturated fatty acids in cellular membranes.[49,50] Hydrogen peroxide (H_2O_2) and oxygen (O_2) are the two constituents derived from the transformation of superoxide radicals (O_{2-}) in the context of a specific class of metalloenzymes acknowledged as SOD. These enzymes are anticipated to have significant implications for the management of disorders associated with oxidative stress.[40] Glutamate, cysteine, and glycine are the amino acids that make up the tripeptide substance glutathione, accounting for electrophile elimination and free radical scavenging. When the equilibrium between ROS and GSH is upset, biomacromolecules undergo destructive oxidation and chemical alteration, which ultimately leads to cell death.[51] Interestingly, selegiline has been discovered to exhibit sufficient efficacy in preserving the balance between ROS production and the antioxidant role of GSH, including the enzymatic activity of SOD, in isolated PBML. This finding suggests the potential benefits of selegiline for those diagnosed with RA.

CONCLUSION

Conducting comprehensive investigations on these parameters utilizing samples from individuals diagnosed with RA might yield valuable insights into the mechanisms of inflammation in RA that are mediated by free radicals. In brief, the findings of this investigation indicate the effectiveness of selegiline in mitigating oxidative overload in isolated PBML cells activated with PMA in patients with arthritis. This is achieved by the reduction of oxidative free radicals and the enhancement of the antioxidative system. Therefore, it can be concluded that the MAO-B inhibitor selegiline has potential as a non-toxic repurposed medicine for curtailing the pathological effects of oxidative overload in RA. Further investigation is warranted to explore the pharmacological effects of selegiline on additional inflammatory cells, such as neutrophils.

ACKNOWLEDGEMENT

The authors would like to acknowledge GIPS, Girijananda Chowdhury University, Guwahati, Assam, for providing the facilities to conduct all these experiments.

REFERENCES

1. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. Lancet. 2010;376(9746):1094-108. Available from: doi: 10.1016/S0140-6736(10)60826-4.
2. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. J Autoimmun. 2020; 110:102400. Available from: doi: 10.1016/j.jaut.2019.102400.
3. Bala A, Mondal C, Haldar PK, Khandelwal B. Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: clinical efficacy of dietary antioxidants. Inflammopharmacology. 2017;25(6):595-607. Available from: doi: 10.1007/s10787-017-0397-1.
4. Weyand CM, Goronzy JJ. The immunology of rheumatoid arthritis. Nat Immunol. 2021;22(1):10-8. Available from: doi: 10.1038/s41590-020-00816-x.
5. Németh T, Nagy G, Pap T. Synovial fibroblasts as potential drug targets in rheumatoid arthritis, where do we stand and where shall we go? Ann Rheum Dis. 2022;81(8):1055-64. Available from: doi: 10.1136/annrheumdis-2021-222021.
6. Wu J, Li Q, Jin L, Qu Y, Liang BB, Zhu XT, et al. Kirenol inhibits the function and inflammation of fibroblast-like synoviocytes in rheumatoid arthritis in vitro and in vivo. Front Immunol. 2019;10:1304. Available from: doi: 10.3389/fimmu.2019.01304
7. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. Available from: doi: 10.1007/s12291-014-0446-0.
8. Oğul Y, Gür F, Cengiz M, Gür B, Sarı RA, Kızıltunç A. Evaluation of oxidant and intracellular anti-oxidant activity in rheumatoid arthritis patients: in vivo and in silico studies. Int Immunopharmacol. 2021;97:107654. Available from: doi: 10.1016/j.intimp.2021.107654.
9. Jing W, Liu C, Su C, Liu L, Chen P, Li X, et al. Role of reactive oxygen species and mitochondrial damage in rheumatoid arthritis and targeted drugs. Front Immunol. 2023;14:1107670. Available from: doi: 10.3389/fimmu.2023.1107670.
10. Wang X, Fan D, Cao X, Ye Q, Wang Q, Zhang M, et al. The role of reactive oxygen species in the rheumatoid arthritis-associated synovial microenvironment. Antioxidants (Basel). 2022;11(6). Available from: doi: 10.3390/antiox11061153.
11. Milkovic L, Cipak Gasparovic AC, Cindric M, Mouthuy PA, Zarkovic N. Short overview of ROS as cell function regulators and their implications in therapy concepts. Cells. 2019;8(8):793. Available from: doi: 10.3390/cells8080793.
12. Quiñonez-Flores CM, González-Chávez SA, Del Río Nájera D, Pacheco-Tena C. Oxidative stress relevance in the pathogenesis of the rheumatoid arthritis: A systematic review. BioMed Res Int. 2016;2016:6097417. Available from: doi: 10.1155/2016/6097417.
13. de Brito Rocha S, Baldo DC, Andrade LEC. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Adv Rheumatol. 2019;59(1):2. Available from:

doi: 10.1186/s42358-018-0042-8.
14. Radu AF, Bungau SG. Management of rheumatoid arthritis: an overview. Cells. 2021;10(11). Available from: doi: 10.3390/cells10112857.
15. Shams S, Martinez JM, Dawson JRD, Flores J, Gabriel M, Garcia G, et al. The therapeutic landscape of rheumatoid arthritis: current state and future directions. Front Pharmacol. 2021;12:680043. Available from: doi: 10.3389/fphar.2021.680043.
16. Watanabe R, Okano T, Gon T, Yoshida N, Fukumoto K, Yamada S, et al. Difficult-to-treat rheumatoid arthritis: current concept and unsolved problems. Front Med (Lausanne). 2022;9:1049875. Available from: doi: 10.3389/fmed.2022.1049875.
17. Boytsov NN, Bhattacharya R, Saverno K, Dixon L, Abbott PL, Zhang X, et al. Health care effect of disease-modifying antirheumatic drug use on patients with rheumatoid arthritis. J Manag Care Spec Pharm. 2019;25(8):879-87. Available from: doi: 10.18553/jmcp.2019.25.8.879.
18. Jost WH. A critical appraisal of MAO-B inhibitors in the treatment of Parkinson's disease. J Neural Transm (Vienna). 2022;129(5-6):723-36. Available from: doi: 10.1007/s00702-022-02465-w.
19. Wang K, Liu ZH, Li XY, Li YF, Li JR, Hui JJ, et al. Efficacy and safety of selegiline for the treatment of Parkinson's disease: A systematic review and meta-analysis. Front Aging Neurosci. 2023;15:1134472. Available from: doi: 10.3389/fnagi.2023.1134472.
20. Ahmari M, Sharafi A, Mahmoudi J, Jafari-Anarkoli I, Gharbavi M, Hosseini MJ. Selegiline (l-deprenyl) mitigated oxidative stress, cognitive abnormalities, and histopathological change in rats: alternative therapy in transient global ischemia. J Mol Neurosci. 2020;70(10):1639-48. Available from: doi: 10.1007/s12031-020-01544-5.
21. Tábi T, Vécsei L, Youdim MB, Riederer P, Szökő É. Selegiline: a molecule with innovative potential. J Neural Transm (Vienna). 2020;127(5):831-42. Available from: doi: 10.1007/s00702-019-02082-0.
22. Ohara A, Iwata N, Sugiura S, Abe N, Nakaseko H, Kawabe S. Evaluation of the European League Against Rheumatism/American College of Rheumatology-2019 classification criteria in patients with childhood-onset systemic lupus erythematosus: a single-center retrospective study. Clin Rheumatol. 2022;41(8):2483-9. Available from: doi: 10.1007/s10067-022-06138-7.
23. Studenic P, Aletaha D, de Wit M, Stamm TA, Alasti F, Lacaille D, et al. American College of Rheumatology/EULAR remission criteria for rheumatoid arthritis: 2022 revision. Ann Rheum Dis. 2023;82(1):74-80. Available from: doi: 10.1136/ard-2022-223413.
24. Suppiah R, Robson JC, Grayson PC, Ponte C, Craven A, Khalid S, et al. American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Microscopic Polyangiitis. Arthritis Rheumatol. 2022;74(3):400-6. Available from: doi: 10.1002/art.41983.
25. Chetia P, Khandelwal B, Haldar PK, Bala A. Dietary antioxidants significantly reduced phorbol myristate acetate induced oxidative stress of peripheral blood mononuclear cells of patients with rheumatoid arthritis. Curr Rheumatol Rev. 2021;17(1):81-7. Available from: doi: 10.2174/1573397116999200729154954.
26. Puleo A, Carroll C, Maecker HT, Gupta R. Isolation of Peripheral Blood Mononuclear Cells Using Vacutainer(®) Cellular Preparation Tubes (CPT(TM)). Bio Protoc. 2017;7(2):e2103. Available from: doi: 10.21769/BioProtoc.2103.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75. Available from: doi: 10.1016/S0021-9258(19)52451-6.
28. Yang J, Cao Y, Zhang N. Spectroscopy spectrophotometric method for superoxide anion radical detection in a visible light (400-780 nm) system. Spectrochim Acta A Mol Biomol Spectrosc. 2020;239(2):118556. Available from: doi: 10.1016/j.saa.2020.118556.
29. Martínez De Toda I, Vida C, Garrido A, De La Fuente M. Redox parameters as markers of the rate of aging and predictors of life span. J Gerontol A Biol Sci Med Sci. 2020;75(4):613-20. Available from: doi: 10.1093/gerona/glz033.
30. Djaballah-Ider F, Touil-Boukoffa C. Effect of combined colchicine-corticosteroid treatment on neutrophil/lymphocyte ratio: a predictive marker in Behçet disease activity. Inflammopharmacology. 2020;28(4):819-29. Available from: doi: 10.1007/s10787-020-00701-x.
31. Devecİ H, Özmen ZC. Evaluation of serum nitric oxide level and its relationship with disease activity parameters in patients with rheumatoid arthritis. J Contemp Med. 2020;10(3):297-301. Available from: doi: 10.16899/jcm.769098.
32. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21(2):130-2. Available from: PMID 6490072.
33. Schneider CD, Bock PM, Becker GF, Moreira JCF, Bello-Klein A, Oliveira AR. Comparison of the effects of two antioxidant diets on oxidative stress markers in triathletes. Biol Sport. 2018;35(2):181–9. Available from: doi: 10.5114/biolsport.2018.74194.
34. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. Available from: doi: 10.1016/0003-2697(79)90738-3.
35. Entezari Heravi N, Mohebbati R, Naji Ebrahimi Z, Khajavi Rad A, Shafei M N, Soukhtanloo M, et al. Effect of Plantago major extract on doxorubicin-induced nephropathy in rat. Physiol Pharmacol 2018; 22 (4) :269-278. Available from: doi: 10.54/pp.2018.269278.
36. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. Available from: doi: 10.1016/0003-9861(59)90090-6.
37. Ahmed OM, Mohammed MT. Oxidative stress: the role of reactive oxygen species (Ros) and antioxidants in human diseases. Plant Arch. 2020;20(2):4089-95. Available from: doi: 10.1549/pa.769458.
38. Mas-Bargues C, Escrivá C, Dromant M, Borrás C, Viña J. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. Arch Biochem Biophys. 2021;709:108941. Available from: doi: 10.1016/j.abb.2021.108941.

39. Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, et al. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. Antioxidants (Basel). 2018;7(8). Available from: doi: 10.3390/antiox7080102.
40. Bassu S, Zinellu A, Sotgia S, Mangoni AA, Floris A, Farina G, et al. Oxidative stress biomarkers and peripheral endothelial dysfunction in rheumatoid arthritis: A monocentric cross-sectional case-control study. Molecules. 2020;25(17):1-11. Available from: doi: 10.3390/molecules25173855.
41. Duarte GBS, Callou KRA, Almondes KGS, Rogero MM, Pollak DF, Cozzolino SMF. Evaluation of biomarkers related to zinc nutritional status, antioxidant activity and oxidative stress in rheumatoid arthritis patients. Nutr Health. 2022;28(2):257-64. Available from: doi: 10.1177/02601060211015594.
42. Da Fonseca LJS, Nunes-Souza V, Goulart MOF, Rabelo LA. Oxidative stress in rheumatoid arthritis: what the future might hold regarding novel biomarkers and add-on therapies. Oxid Med Cell Longev. 2019;2019:7536805. Available from: doi: 10.1155/2019/7536805.
43. Cheng Q, Wu H, Du Y. The roles of small-molecule inflammatory mediators in rheumatoid arthritis. Scand J Immunol. 2021;93(3):e12982. Available from: doi: 10.1111/sji.12982.
44. Sadeghi A, Bastin AR, Ghahremani H, Doustimotlagh AH. The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Mol Biol Rep. 2020;47(5):3557-66. Available from: doi: 10.1007/s11033-020-05447-x.
45. Piacenza L, Zeida A, Trujillo M, Radi R. The superoxide radical switch in the biology of nitric oxide and peroxynitrite. Physiol Rev. 2022;102(4):1881-906. Available from: doi: 10.1152/physrev.00005.2022.
46. Kumar S, Dang S, Nigam K, Ali J, Baboota S. Selegiline nano formulation in attenuation of oxidative stress and upregulation of dopamine in the brain for the treatment of Parkinson's disease. Rejuvenation Res. 2018;21(5):464-76. Available from: doi: 10.1089/rej.2017.2035.
47. Nakai S, Yoneda F. A theoretical investigation of (-)-deprenyl (selegiline) as a radical scavenger. Theoretical Chemistry Accounts: Theory Computation and Modelling. 2000;104(5):398-406. Available from: doi: 10.1007/s002140000161.
48. Ahmed AB, Chetia P. MAO – B Inhibitor "Selegiline" Attenuates Oxidative Stress and Arthritic Parameters in an Invitro Repurposive Study. Journal of Biomedical Engineering. 2023;40(2), 171–181. Available from: doi: 10.105515/JBE.40.2.17
49. Taso OV, Philippou A, Moustogiannis A, Zevolis E, Koutsilieris M. Lipid peroxidation products and their role in neurodegenerative diseases. Ann Res Hosp. 2019;3(4):2. Available from: doi: 10.21037/arh.2018.12.02.
50. Kotnala M, Virmani T. Effectiveness of Anaphalis triplinervis on Carbon Tetrachloride-induced Hepatic Injury in Rats by Interfering with the Inflammatory, Cell Death and Oxidative-stress Pathways. International Journal of Pharmaceutical Quality Assurance. 2022;13(1):42-51. Available from: doi: 10.25258/ijpqa.13.1.10
51. Liu T, Sun L, Zhang Y, Wang Y, Zheng J. Imbalanced GSH/ROS and sequential cell death. J Biochem Mol Toxicol. 2022;36(1): e22942. Available from: doi: 10.1002/jbt.22942.