ATATURK
UNIVERSITY
PUBLICATIONS

Recent Trends in Pharmacology

Offical journal of Atatürk University Faculty of Medicine
Department of Medical Pharmacology

Volume 2• Issue 1 • April 2024

EISSN 2980-194X
https://dergipark.org.tr/en/pub/rtpharma

Recent Trends in Pharmacology

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Zafer SEZER
Department of Medical Pharmacology,
Erciyes University, Kayseri, Türkiye
Erciyes Üniversitesi-Tıbbi Farmakoloji Bölümü, Kayseri, Türkiye

Nuri İhsan KALYONCU
Department of Medical Pharmacology,
Karadeniz Tehcnical University, Trabzon, Türkiye
Karadeniz Teknik Üniversitesi-Tıbbi Farmakoloji Bölümü, Trabzon, Türkiye

Hakkı Zafer GÜNEY
Department of Medical Pharmacology,
Lokman Hekim University, Ankara, Türkiye
Lokman Hekim Üniversitesi-Tıbbi Farmakoloji Bölümü, Ankara, Türkiye

Mehmet Yıldırım SARA
Department of Medical Pharmacology,
Hacettepe University, Ankara, Türkiye
Hacettepe Üniversitesi-Tıbbi Farmakoloji Bölümü, Ankara, Türkiye

Filiz ONAT
Department of Medical Pharmacology,
Acıbadem Mehmet Ali Aydınlar University, İstanbul, Türkiye
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Kayseri, Türkiye

Ahmet KIZILTUNÇ
Department of Medical Biochemistry,
Atatürk University, Erzurum, Türkiye
Atatürk Üniversitesi-Tıbbi Biyokimya Bölümü, Erzurum, Türkiye

Hasan TÜRKEZ
Department of Medical Biology,
Atatürk University, Erzurum, Türkiye
Atatürk Üniversitesi-Tıbbi Biyoloji Bölümü,
Erzurum, Türkiye

Abdülgani TATAR
Department of Medical Genetics,
Atatürk University, Erzurum, Türkiye
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Demet ÇELEBİ
Department of Medical Microbiology,
Atatürk University, Erzurum, Türkiye
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Erzurum, Türkiye

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Department of Medical Pathology,
Atatürk University, Erzurum, Türkiye
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M. Sait ERTUĞRUL
Department of Food,
Ondokuz Mayıs University, Erzurum, Türkiye
Ondokuz Mayıs Üniversitesi-Gıda Bölümü,
Erzurum, Türkiye

Cemil BAYRAM
Department of Pharmacology and Toxicology,
Atatürk University, Erzurum, Türkiye
Atatürk Üniversitesi-Farmakoloji ve Toksikoloji Bölümü, Erzurum, Türkiye

İ. Çağrı AYDIN
Department of Pharmacology,
Erzincan Binali Yıldırım University, Erzincan, Türkiye
Erzincan Binali Yıldırım *Üniversitesi-Farmakoloji Bölümü, Erzincan, Türkiye*

Mehmet Ali YÖRÜK
Department of Pharmacology and Toxicology,
Atatürk University, Erzurum, Türkiye
Atatürk Üniversitesi-Farmakoloji ve Toksikoloji Bölümü, Erzurum, Türkiye

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Recent Trends in Pharmacology is a peer-reviewed, open-access, online-only journal published by Atatürk University.
The journal is published triannual in both English, with articles released in April, August, and December.
All expenses of the journal are covered by the Atatürk University. Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at https://dergipark.org.tr/en/pub/rtpharma. The journal guidelines, technical information, and the required forms are available on the journal's web page.

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Contact (Editor in Chief) / İletişim (Baş Editör)

Ahmet HACIMÜFTÜOĞLU
Atatürk University, Faculty of Medicine, Erzurum, Turkey
Atatürk Üniversitesi, Tıp Fakültesi, Erzurum, Türkiye
ahmeth@atauni.edu.tr
ataunijournals@atauni.edu.tr
https://dergipark.org.tr/tr/pub/rtpharma

Contact (Publisher) / İletişim (Yayıncı)

Atatürk University
Atatürk University, Erzurum, Turkey
Atatürk Üniversitesi Rektörlüğü 25240 Erzurum, Türkiye
ataunijournals@atauni.edu.tr
https://bilimseldergiler.atauni.edu.tr
+90 442 231 15 16

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Research Article DOI: 10.62425/rtpharma.1466682

Antitumoral Effect of Syringic Acid on DU-145 Prostate Cancer Cells Antitumoral Effect of Syringic Acid

Yeşim YENİ[1]
Sıdıka GENÇ[2]
[1]Department of Medical Pharmacology, Faculty of Medicine, Malatya Turgut Özal University, Malatya, Türkiye
[2]Department of Medical Pharmacology, Faculty of Medicine, Bilecik Seyh Edebali University, Bilecik, Türkiye

ABSTRACT

Objective: Prostate cancer (PC) is one of the most prevalent reasons for cancer-killing in men worldwide, and new drugs to treat PC are currently being developed. Syringic acid (SA) is a polyphenolic compound that exhibits anti-inflammatory and metabolic regulatory effects and antitumor activities in various tumors. This study purposed to research the antiproliferative and antitumor activities of SA on DU-145 cells.

Methods: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as used to assign the antiproliferative effect of SA and Superoxide dismutase (SOD)- Malondialdehyde (MDA) analyses were used to determine its antioxidant-oxidant effects.

Results: SA significantly suppressed DU-145 cell proliferation in vitro. Additionally, while it reduced SOD levels, it caused a significant increase in MDA levels.

Conclusion: Our findings revealed the antitumor potential for PC by targeting the curative effect of SA.

Keywords: DU-145, Malondialdehyde, Prostate cancer, Superoxide dismutase, Syringic acid

Received 18.03.2024
Accepted 22.04.2024
Publication Date 03.05.2024

Corresponding author:
Yeşim Yeni
E-mail: yesim.yeni@ozal.edu.tr
Cite this article: Yeni, Y., & Genç, S. (2024). Antitumoral Effect of Syringic Acid on DU-145 Prostate Cancer Cells Antitumoral Effect of Syringic Acid. *Recent Trends in Pharmacology, 2*(1), 1-5.

Introduction

According to World Health Organization GLOBOCAN data, prostate cancer (PC) is the most prevalent type of cancer in men worldwide. It is the 2nd most prevalent cause of death after lung cancer (Siegel et al., 2022). In studies conducted in Türkiye, it is the 3rd most prevalent type of cancer in men and the 4th in cancer-based kills (Sung et al., 2021). PC is more strongly associated with age than other known types of cancer (Jemal et al., 2021). Prostate adenocarcinoma is a heterogeneous group of diseases, and while it has a silent course in some patients, it can have an aggressive course in others (Harlan et al., 2001). Today, androgen-deprived therapies and docetaxel, abiraterone acetate, and enzalutamide-related chemotherapy are standard therapies for forward PC patients (Cornford et al., 2021; Cattrini et al., 2019). However, traditional chemotherapy can with easy reason important side effects in the hematopoietic tract, repeat drug withdrawal reactions, and severe drug resistance, placing a heavy load on patients. For this reason, it is important to research new and effective drugs for PC metastasis and proliferation (Petrylak et al., 2004).

Natural products have been demonstrated to be the most inevitable resource of anticancer drugs like paclitaxel and vincristine, which have succeeded greatly in the clinical therapy of diverse cancers (Kingston, 2009). Natural products generally have lower toxicity and higher efficacy than traditional chemotherapy drugs. In the process of researching drugs for the treatment of PC, various active materials obtained from natural resources have been demonstrated to have anticancer potential (Fontana et al., 2020). SA has shown that it can modulate signaling molecules, transcriptional factors, proteins, and growth factors, especially in diverse cancer cells (Srinivasulu et al., 2018; Rob et al., 2020). SA has diverse physiological functions like anti-oxidant, anti-diabetic, anti-inflammatory, hepatoprotective, anti-cancer, and anti-mitogenic properties (Srinivasulu et al., 2018). Furthermore, research on SA's cytotoxic effect in colorectal, breast, and lung cancer cell lines has produced promising results (Gheena & Ezhilarasan, 2019). Numerous extracts and several plants that contain SA have established anti-inflammatory activities (Ham et al., 2016; Tanaka et al., 2017). Scientific literature suggests that SA may be therapeutic on A549 lung cancer cells (Karthick et al., 2014). Considering the desire to improve recent anti-cancer agents, herb-related bioactive phytochemicals present loud efficiency and low toxicity properties that can ultimately be used in the clinical therapy of PC patients. For this reason, in this work, we researched whether SA exerts any effect on PC cells.

Methods

Cell Culture

The DU-145 cell line was incubated with a medium containing 15% Fetal Calf Serum, Eagle's Minimum Essential Medium, and the antibiotic penicillin-streptomycin (Sigma, USA) at 37°C, 5% CO2, 95% humidity and 25-75 cm2 cell culture flasks. After sufficient consolidation, the 75 cm2 bottle was washed with phosphate-buffered saline (Sigma, USA). Then, the cells were separated from the vial by adding Tyripsin-EDTA (Sigma, USA) and after centrifugation, the cells were transferred to 96-well plates. The cell culture dose of SA was determined with reference to previous studies (Gheena & Ezhilarasan, 2019). When the cells covered approximately 85% of the well surface, SA was applied to the wells at concentrates of 5-10-20- 40, and 80 µg/mL. After 24 hours, cell viability was measured.

Cell Viability

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Sigma, USA) test was used to appraise cell viability. 10 µL of MTT resolution was supplemented to all wells and after the incubation period, the formazan crystals were dissolved in 100 µl of dimethyl sulfoxide (Sigma, USA). Finally, the absorbance value was determined at 570 nm in the spectrophotometer (BioTek, USA) (Yeni et al., 2023).

Biochemical Analysis

Cell medium was gathered 1 day after toxicity administration and assayed according to the manufacturer's instructions.

Malondialdehyde (MDA) levels MDA ELISA kit (E-BC-K025-S; Elabscience, USA) was used in the evaluation and the method was performed as in the instructions. Optical density was measured spectrophotometrically at 450 nm wavelengths. MDA activity was stated as nmol/mg Protein.

Superoxide Dismutase (SOD) levels SOD ELISA kit (E-EL-H1113; Elabscience, USA) was used in the evaluation and the method was performed as in the instructions. Optical density was measured spectrophotometrically at 450 nm wavelengths. SOD activity was stated as pg/mL.

Statistical Analysis

Data were analyzed with the GraphPad 8.02 program.

ELISA results were analyzed using the One-way analysis of variance analysis of the variance test. For post-hoc analysis, the Dunnett test was performed.

Results

Cell Viability

The MTT test indicated the cytotoxicity results for all groups 24 hours after application. Figure 1 shows the cytotoxicity of SA at various doses (5-80 µg/mL) against PC cells. Cell viability was considered as 100 in the control group. In the SA group, this rate was shown to be lower than in the control group. No significant results were obtained in SA 5, 10, and 20 µg/ml groups. However, viability was significantly affected at high concentrations. Cell viability significantly was reduced in SA 40 and 80 µg/ml group (p<0.01). A nearly 20% and 40% reduction in this viability verifies the cytotoxic effect of in SA 40 and 80 µg/ml group, respectively.

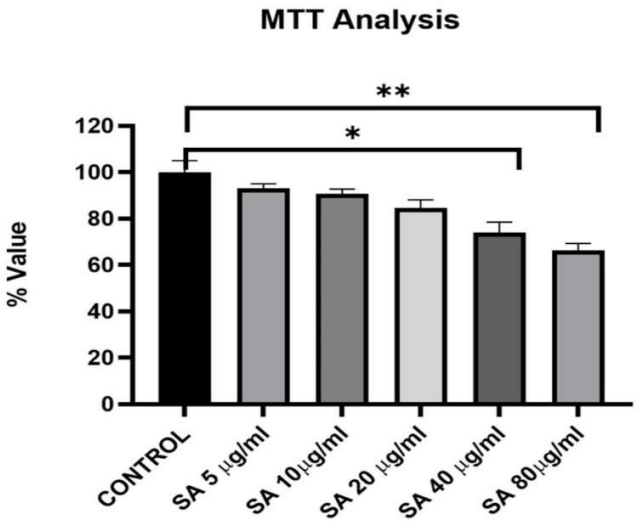

Figure 1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) results of the application group. Data are stated as the means ± SD. *p<0.05, **p<0.01 values are very significant for the control group.

SOD-MDA

For samples treated with 40 and 80 µg/ml SA, ROS levels dramatically rose (p<0.01) (Figure 2), whereas oxidative stress in antioxidant enzymes (SOD) decreased, showing the production of ROS (Figure 2). The identification is caused by two methoxy groups linked to the aromatic ring of an SA molecule at positions 3 and 5. SOD level was determined as 44.72 pg/ml in the control group. Upon treatment with the SA group, cell viability began to decrease. Thus, the resulting redox parameters began to affect cell viability. In the SA 40 and 80 µg/ml groups, along with the decrease in cell viability, there was also a severe decrease in sod activities. This value was determined in the SA 80 µg/ml group as 15.97 pg/ml (p<0.01).

Oxidative damage in the cell was determined by MDA. Interestingly, when ROS levels in cells were evaluated after exposure to SA, ROS production was found to increase in a concentration-dependent manner like chemotherapeutics. (Figure 2). However, the ROS level was significantly higher in the 80ug/ml group (25.80 nmol/mg protein) (p < 0.01).

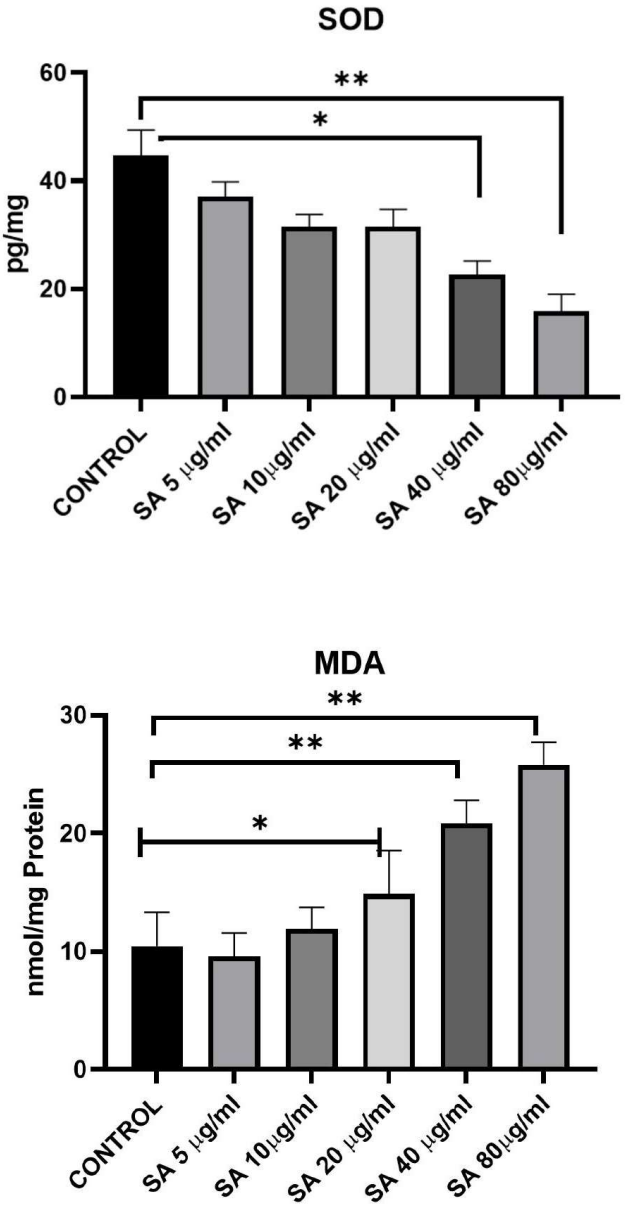

Figure 2. Superoxide dismutase (SOD) and

Malondialdehyde (MDA) results of application group. The values have been displayed as mean ± SD. *p<0.05, **p<0.01 values are very significant for the control group.

Discussion

Cancer is an illness with cell loop irregularity (Park & Lee, 2003). Numerous works have shown that natural products and their simultaneous therapy and chemotherapy are effective in malignant tumors. Active materials extracted from natural products can modulate the cancer microenvironment and various cell signaling cascades, so they play an important role in fighting cancer (Fontana et al., 2020; Dutta et al., 2019; Salehi et al., 2019). SA can stimulate apoptosis in diverse cancer cells (Srinivasulu et al., 2018). It has recently been reported that SA exhibits cytotoxicity features against oral squamous cell carcinoma SCC131 (Velu et al., 2020). In this study, SA depressed the growth of PC cells in a concentrated-dependent manner and demonstrated that the count of dead cells increased in SA-therapy cells. In the present study, the status of antioxidant enzymes in PC cells showed reactive oxygen species accumulation in SA-treated cells. Here we report that SA causes a significant inhibitory effect on PC cell metastasis and growth.

One study showed that treatment with SA led to an increase in the count of ROS-produced SW-480 cell populations compared to controls. More significantly, evaluation of the antioxidant ingredient of the cells demonstrated that SA caused a decrease in the level of antioxidant enzymes like CAT, GST, GPx, GR, and SOD, in tumor cells. For this reason, antioxidant consumption, which has recently been associated with cell killing, is another mechanism for the antitumor effects of SA in colorectal tumor cells (Mihanfar et al., 2021). Besides in vitro works, the antitumor effects of SA have been verified in animal models including diverse types of cancer. In a work conducted on rats, the chemo-protective effects of SA were investigated. A preadministration concentrate of 50 mg/kg was able to decrease the abnormal expression of cytokeratin and positively modulate cell surface glycoconjugates. The findings were also verified in histological investigation; because those pretreated had standard histological results compared with those exposed to the carcinogen alone. Some of these effects were found to be partly mediated by the antioxidant role of SA (Periyannan & Veerasamy, 2018). The incorporation of SA into gastric cancer cells has been shown to inhibit the advancement of inflammatory mediators mainly through regulation of the AKT/mTOR signaling path (Pei et al., 2021). For this reason, SA may be a candidate agent for use in PC treatment with its anti-inflammatory effect.

Conclusion

Studies show the antioxidant, anti-inflammatory, anti-lipid peroxidative, and anticancer effects of SA on PC. This investigation supports the evidence showing the curative effect of SA on PC. Therefore, SA is waiting to serve as a new anticancer drug in the therapy of PC.

Ethics Committee Approval: Since the cell line was studied in vitro, an ethics committee decision is not required.
Informed Consent: Since it is an in vitro study, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – YY; Design - YY; Supervision – YY, SG; Resources – YY; Materials – YY, SG; Data Collection and/or Processing – YY, SG; Analysis and/or Interpretation – YY, SG; Literature Search – YY, SG; Writing Manuscript – YY, SG; Critical Review – YY, SG; Other – YY
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

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Research Article DOI: 10.62425/rtpharma.1470761

Exploring the Therapeutic Effects of *Radix astragalus* on Glioblastoma Multiforme Cell Culture

Irmak FERAH OKKAY[1]
Ufuk OKKAY[2]

[1]Department of Pharmacology, Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye
[2]Department of Medical Pharmacology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye

ABSTRACT

Objective: This study investigated the effectiveness of *Radix astragalus*, a traditional herbal remedy, in combating Glioblastoma Multiforme (GBM), a highly aggressive brain tumor. Current therapies for GBM are limited in their efficacy, highlighting the urgent need for innovative treatment strategies.

Methods: Our research employed advanced methods to assess the anti-tumor properties of *Radix astragalus* extracts on GBM cell lines in vitro. We evaluated cell viability and proliferation using MTT and LDH assays. Furthermore, we analyzed the oxidative stress levels within GBM cells by measuring Total Antioxidant Capacity (TAC), Total Oxidant Status (TOS), and malondialdehyde (MDA) values. These comprehensive techniques provided valuable insights into the intricate interactions between GBM cells and the extracts.

Results: The results were highly promising. *Radix astragalus* extracts significantly reduced GBM cell viability, demonstrating a potent antitumorigenic effect. Additionally, the extracts effectively countered the oxidative stress within GBM cells, a key factor promoting tumor growth and progression. Moreover, antioxidant assays revealed enhanced antioxidant activity in GBM cells treated with *Radix astragalus* compared to untreated controls.

Conclusion: These findings unveil the remarkable potential of *Radix astragalus* as a novel therapeutic approach for GBM treatment. The extract's ability to target both GBM cell viability and oxidative stress offers a promising avenue for future cancer research. Further investigations are warranted to explore the full potential of *Radix astragalus* in developing effective therapies for this challenging form of brain cancer.

Keywords: GBM, *Radix astragalus*, Anticancer

Received 22.03.2024
Accepted 02.05.2024
Publication Date 03.05.2024

Corresponding author:
Ufuk Okkay
E-mail: ufukokkay@atauni.edu.tr
Cite this article: Ferah Okkay, I., Okkay, U. (2024). Exploring the Therapeutic Effects of *Radix astragalus* on Glioblastoma Multiforme Cell Culture. *Recent Trends in Pharmacology, 2*(1), 6-10.

Introduction

Glioblastoma multiforme (GBM), often referred to as glioblastoma, is the most common primary malignant brain tumor with an incidence of approximately 3.2 per 100,000 people per year in the United States. It is thought to have its genesis in neuroglial stem or progenitor cells (Le Rhun et al., 2019). Despite the utilization of a range of sophisticated therapeutic modalities, including surgical intervention, radiotherapy, chemotherapy, targeted therapy and supportive care, the median survival time of the majority of patients diagnosed with glioblastoma multiforme (GBM) remains below 15 months, with a 5-year survival rate of less than 3% (Fisher & Adamson, 2021). Despite the availability of numerous treatment options, GBM remains a formidable challenge due to its highly invasive nature and the limited efficacy of current therapies. Although surgical intervention followed by adjuvant radiation and chemotherapy can prolong survival, the overall prognosis for patients with GBM remains dismal, with most patients experiencing recurrence and disease progression. Consequently, there is an immediate requirement to identify novel therapeutic targets and develop more effective treatment strategies in order to enhance patient outcomes. The use of temozolomide, an orally available alkylating agent, has demonstrated some benefits in the treatment of GBM (Wang et al., 2023). However, the emergence of chemoresistance has limited its effectiveness.

The development of new drugs with alternative mechanisms of action is critical to overcome chemoresistance and improve survival outcomes. Additionally, targeted therapies that specifically target GBM cells while sparing healthy brain tissue may provide a more effective and less toxic alternative to traditional chemotherapy. It is therefore imperative to identify and develop innovative treatment options for GBM. Herbs have been utilized by humans for at least 60,000 years to treat various diseases, indicating their diverse pharmacological properties, including anti-cancer activity (Wang et al., 2020). Herbal formulations can act on multiple targets through their various constituents, thereby playing a crucial role in important biological processes and exhibiting therapeutic effects during the progression of diseases (Yang et al., 2018). One such traditional Chinese medicine is *Radix astragalus* (RA), several studies have reported the antitumor activity of RA. It has been demonstrated that RA extracts are capable of inhibiting the growth of lung adenocarcinoma cells (Wei et al., 2020). Zhang et al. demonstrated that the laryngeal carcinoma SCC15 cell line exhibited concentration-dependent antitumor activity following treatment with total RA glucosides (Wang et al., 2020). The multi-targeted action and diverse constituents of RA make it an attractive option for treating various diseases, including cancer (Guo et al., 2019). The antitumor activity of RA may be attributed to its ability to regulate key signaling pathways involved in cancer development and progression. Thus, further investigation of the mechanisms underlying RA's antitumor activity is warranted to fully understand its therapeutic potential in cancer treatment.

Considering the potential therapeutic benefits of RA in cancer treatment, we aimed to investigate its effect on GBM and elucidate its underlying molecular mechanism. The objective of this study was to provide a scientific foundation for future research on the potential therapeutic use of RA in the treatment of GBM.

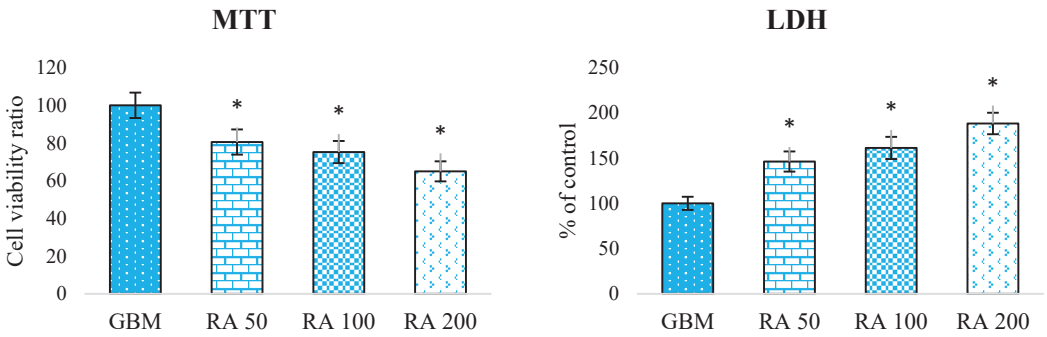

Figure 1. Cell viability test results.

Methods

Cell culture

GBM cells were used in the studies for anticancer activity. The cells were incubated in RPMI 1640 medium containing penicillin (100 units/mL), streptomycin (100

µg/mL), fetal bovine serum (10%), and L-glutamine (2.5 mM) at 37 °C with a humidified atmosphere of 5% CO2 and 95% air. The culture medium was replaced on three occasions each week.

MTT assay

In order to assess the cytotoxicity of RA, the MTT assay was conducted in triplicate on GBM cells. The cells were cultivated in 96-well plates with a capacity of 5 × 103 cells per well. They were subjected to a 24-hour treatment with RA at doses of 50 µg/mL, 100 µg/mL, and 200 µg/mL. After that, each well received 20 µL of MTT solution, and the plate was covered with foil and incubated at 37°C for two hours. After that, the MTT solution was discarded, and 150 µL of dimethylsulfoxide (DMSO) was used to dissolve the blue-violet formazan that had developed in the wells. The plate was incubated under light-deprived conditions for a duration of 30 minutes, following which the optical density values were measured at a wavelength of 570 nm employing an Elisa Reader (Ferah Okkay et al., 2021). Untreated cells were employed as a negative control for assessing cytotoxicity, indicative of 100% mitochondrial function.

Lactic dehydrogenase release

To evaluate cell death resulting from membrane disruption, the release of lactic dehydrogenase (LDH) was assessed. The LDH activity was determined through spectrophotometry analysis in the culture medium at a wavelength of 340 nm, focusing on the reduction of nicotinamide adenine dinucleotide (NAD) (Birdal et al., 2024). The percentage of LDH release was computed as the proportion of the total quantity found in both the cellular lysate and the culture medium. The findings were presented in terms of the percentage of LDH that was released.

Measurement of oxidative burden

The assessment of antioxidative capacity within cell culture supernatants was conducted through the employment of automated methodologies measuring total antioxidant capacity (TAC) and total oxidant status (TOS) utilizing commercially available kits provided by Rel Assay Diagnostics®. In order to ascertain the TAC levels, the specimens underwent treatment with Reagent 1 followed by the measurement of absorbance at 660 nm (Okkay et al., 2021). After the addition of Reagent 2 and incubation, the absorbance was read again to measure the reduction of ABTS radicals. The TOS levels were assessed through the treatment of the samples with Reagent 1 followed by the measurement of absorbance at 530 nm. The introduction of Reagent 2 resulted in a chromogenic reaction within the solution, and the intensity of the color was quantified using spectrophotometric method. Both TAC and TOS analyses were performed in 48-well plates using kit standards

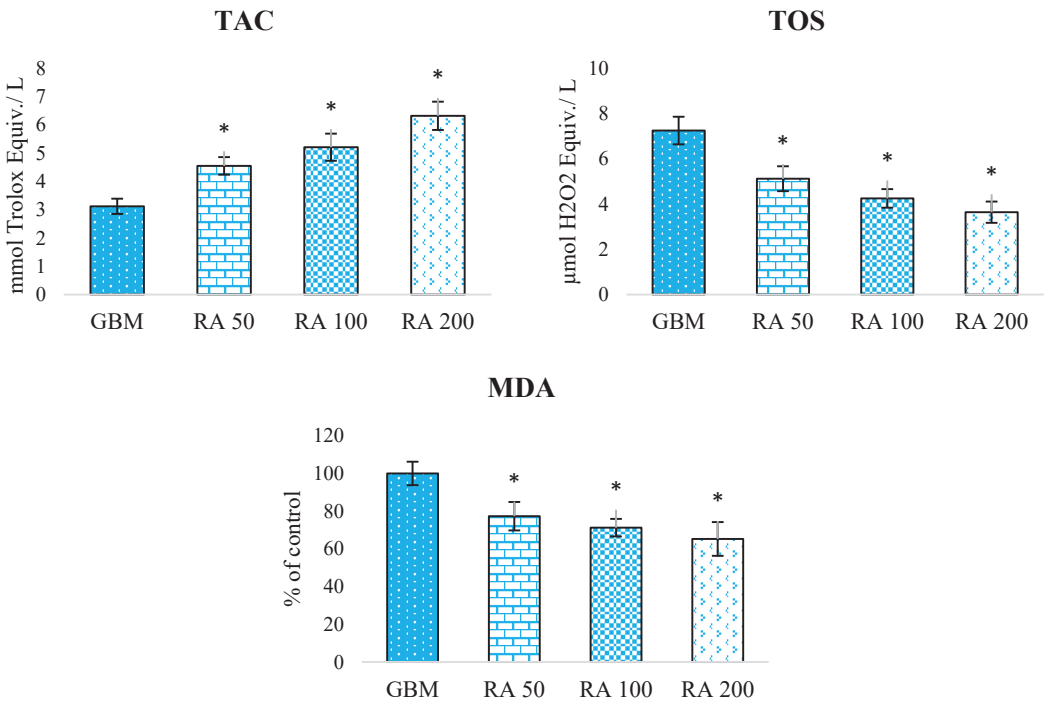

Figure 2. Oxidative stress analysis results.

(Trolox 1 mmol/L and H202 10 µmol/L, respectively) and dH2O as controls. The results were read in a plate reader and interpreted according to the manufacturer's instructions.

Statistical analysis

Using IBM SPSS 22.0, one-way analysis of variance (ANOVA) with post hoc Tukey's test ($p < 0.05$) was used for all analyses. The data was shown as mean ± SD.

Results

Cell viability

The colorimetric MTT test was employed to determine the percentage of GBM cell line viability. To assess the effectiveness of RA, GBM cells were treated with various concentrations of RA for 24 hours. Results are presented in Fig. 1. The optimal concentration and duration of RA treatments were found to be 200 µg/ml, which significantly inhibited cell growth in a dose- and time-dependent manner compared to the control group ($P < 0.05$).

Measurement of oxidative burden

The study found that the TAC levels in the RA group were significantly higher ($p < 0.05$) compared to the GBM group. This means that the RA group had a higher overall antioxidant capacity, which may indicate a lower oxidative burden. On the other hand, the TOS and MDA values in the RA group were significantly lower ($p < 0.05$) compared to the GBM group. This suggests that the RA group had a lower overall level of oxidative stress.

To visually represent these findings, the study included a figure (Fig. 2) which presumably shows the TAC, TOS and MDA levels for both groups. Based on the results, we can infer that the TAC levels for the RA group are higher than the GBM group, while the TOS and MDA levels for the RA group are lower than the GBM group.

Discussion

GBM is a remarkably aggressive and fatal primary neoplasm of the brain, demonstrating resistance towards traditional therapeutic modalities including chemotherapy and radiotherapy. (Davis, 2016). Hence, it is imperative to discern novel and efficacious therapeutic strategies for GBM. This investigation delves into the plausible therapeutic impacts of RA on GBM cell populations. The outcomes of our research indicate that RA significantly diminishes the survival rate of GBM cells, as assessed through MTT and LDH analyses. This suggests that RA may have cytotoxic effects on GBM cells, which could be explored further in future studies.

Moreover, it was noted that RA resulted in an elevation of the overall antioxidant potential while concurrently reducing the overall oxidative status in GBM cells. These findings suggest that RA may have anti-oxidant properties that could potentially counteract the oxidative stress that is often observed in cancer cells. Our findings align with prior research studies that have documented the anti-cancer properties of RA across a range of cancer types such as breast, gastric, and lung cancer (Li et al., 2020; Wu et al., 2018). Previous literature indicates that the mechanisms responsible for the anti-cancer effects of RA are intricate, involving the regulation of diverse signaling cascades, including the PI3K/Akt/mTOR pathway, the NF-κB pathway, and the JAK/STAT pathway (Zhou et al., 2018).

Despite the encouraging findings of our research, it is important to acknowledge certain constraints. Initially, the scope of our investigation was limited to examining the impact of RA on GBM cells in a controlled laboratory setting, necessitating additional research to validate its therapeutic efficacy in live subjects. Second, the mechanisms underlying the cytotoxic and anti-oxidant effects of RA on GBM cells are still unclear and require further investigation.

Conclusion

In conclusion, our study provides evidence that RA has potential therapeutic effects on GBM cells. The cytotoxic and anti-oxidant effects of RA on GBM cells suggest that it may have a role as an adjunctive therapy for GBM. Future research endeavors should focus on elucidating the mechanisms that underlie the anti-tumor properties of RA, as well as exploring its potential as a therapeutic intervention for GBM.

A part of this study was presented as an oral presentation at the **3rd International Black Sea Modern Scientific Research Congress**.

Ethics Committee Approval: Since the cell line was studied in vitro, an ethics committee decision is not required.
Informed Consent: Since it is an in vitro study, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - UO, IFO; Design- UO, IFO; Supervision- UO, IFO; Resources- UO, IFO; Data Collection and/or Processing- UO, IFO; Analysis and/or Interpretation- UO, IFO; Literature Search- UO, IFO; Writing Manuscript- UO, IFO; Critical

Review- UO, IFO.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article DOI: 10.62425/rtpharma.1462458

Changes in Smoking Behavior in the COVID-19 Pandemic: A Single Center Study in Family Practice

Kamber KAŞALİ[1]
Mehmet DOĞAN[2]
Hacı Ahmet AYDEMİR[3]
Mustafa BAYRAKTAR[4]

[1]Department of Biostatistics, Atatürk University, Medical Faculty, Erzurum, Türkiye
[2]Şefaatli Family Health Center, Yozgat, Türkiye
[3]Filiz Dolunay Family Health Center, Erzurum, Türkiye
[4]Department of Family Medicine, Atatürk University, Medical Faculty, Erzurum, Türkiye

ABSTRACT

Objective: There are controversial studies on smoking addiction in the COVID-19 pandemic. While some studies show increased tobacco use during the pandemic, others report increased smoking cessation attempts. This study examined changes in tobacco use and quit intentions during the COVID-19 pandemic compared to pre-pandemic.

Methods: A cross-sectional study was designed and three groups were formed by including patients over 18 years of age, who were smokers, and who presented to the Education Family Health Center and had RT-PCR test results for COVID-19. Among these patients, patients with COVID-19 symptoms and complaints and positive RT-PCR test results constituted the COVID-19 positive group, patients who were COVID-19 negative but isolated due to their contacts constituted the contact group, and healthy people without any COVID-19 contact and symptoms constituted the healthy group. Demographic characteristics, smoking status before the COVID-19 pandemic, change in smoking status after COVID-19 test result or contact, and Fagerström nicotine dependence test results were compared.

Results: Of the total 131 participants enrolled in the study, 70 were in the healthy group, 31 were in the COVID-19 negative group, and 30 were in the COVID-19 positive group. Men were predominant in three groups (60%, 83.9%, and 73.3% in normal, COVID-19-neg, and COVID-19-positive groups, respectively) with a small significant difference ($p=0.048$). The mean ages were 41.09±12.85, 38.21±11.69, and 39.47±11.66 years in the healthy, COVID-19 negative, and COVID-19 positive groups, respectively, with no significant difference ($p>0.05$). Fagerström dependence scores were 1.82±1.05, 1.53±0.86, and 1.40±0.72 in the healthy, COVID-19 negative, and COVID-19 positive groups, respectively, and were not statistically different ($p>0.05$). Smoking prevalence decreased by 1.13 (±4.17) cigarettes per day in the healthy group, by 3.97 (±5.31) cigarettes in the COVID negative group, and by 10.14 (±7.86) cigarettes in the COVID positive group, with a statistically significant decrease in smoking prevalence in all three groups ($p<0.01$). Those in the COVID-19 positive group were statistically significantly more likely to want to stop smoking.

Conclusion: Our study found that people significantly reduced smoking and considered quitting during the COVID-19 pandemic. Every individual who quits smoking is an asset to society. To achieve this goal, it should be aimed at raising awareness and providing counseling to quit smoking by studying smoking and nicotine dependence in all situations.

Keywords: COVID-19, Family practice, Nicotine dependence, Smoking

Received 01.04.2024
Accepted 02.05.2024
Publication Date 03.05.2024

Corresponding author:
Mustafa BAYRAKTAR
E-mail: mustafabayraktar@atauni.edu.tr
Cite this article: Kaşali, K., Doğan, M., Aydemir HA., & Bayraktar, M. (2024). Changes in Smoking Behavior in the COVID-19 Pandemic: A Single Center Study in Family Practice. *Recent Trends in Pharmacology, 2*(1), 11-20.

Introduction

Smoking is known to negatively affect the immune system and reduce the defense against infectious diseases. The negative effects of smoking on the respiratory tract and lungs have been shown to have a negative impact on the prognosis of COVID-19. In most studies on the mortality of COVID-19, smoking has been shown to increase the mortality and complications of the disease (Berlin et al., 2020; Eisenberg & Eisenberg, 2020; Farsalinos et al., 2020; Roengrudee Patanavanich & Stanton A Glantz, 2020). Among patients with COVID-19, it is known that smokers and former smokers need intensive care and mechanical ventilation more than non-smokers. In addition, severe COVID-19 symptoms were 1.4 times more common in former smokers and current smokers compared to non-smokers (Freire et al., 2022).

Adverse effects of smoking on COVID-19 infection have inevitably affected people's smoking behavior. However, the effects of the COVID-19 pandemic on smoking addiction are controversial. While tobacco use has increased in some countries since the beginning of the pandemic, tobacco cessation attempts and the success of these attempts have also increased (Veldhuizen et al., 2021).

Changes in smoking behavior and quit attempts appear to be related to the socially restrictive measures taken to prevent the spread of the disease. The strict social isolation measures taken to prevent the spread of COVID-19 have led to the emergence of severe negative psychological symptoms. Smoking also appears to have increased to relieve these psychological symptoms (García-Álvarez et al., 2020; Ornell et al., 2020).

In the COVID-19 pandemic, restricted social life, job losses, remote working (Veldhuizen et al., 2021), the claim that smoking may be protective against the virus in COVID-19 (Changeux et al., 2020; Paleiron et al., 2021; van Zyl-Smit et al., 2020) and coronaphobia (Ozcelik & Yilmaz Kara, 2021) seem to have caused a change in tobacco use behavior and quitting behavior (Ozamiz-Etxebarria et al., 2020; Ozcelik & Yilmaz Kara, 2021; van Zyl-Smit et al., 2020; Veldhuizen et al., 2021). Differences in health service delivery also seem to be effective on this change. With the restrictive measures taken to reduce transmission, health service delivery has also started to be provided virtually. Health services provided virtually seem to be effective on tobacco cessation behavior and patients in tobacco cessation programs (Veldhuizen et al., 2021).

The emotional aspect of the issue cannot be ignored. At this point, studies showing that there is an increase in the number of former smokers who start smoking again after national disasters and an increase in the number of current smokers support this emotional burden (Lanctot et al., 2008; Osaki et al., 2020; Parslow & Jorm, 2006; Vlahov et al., 2002). Interest in smoking cessation has also increased following these national disasters, along with increased use (Lanctot et al., 2008).

As can be seen, it has not been clearly determined how smoking will be affected in the pandemic. Studies seem to have focused more on the effects of the association of smoking and COVID-19 infection on complications and mortality. And this negative association seems clear at this point. This negative association between smoking and COVID-19 creates an expectation that there will be an increase in smoking cessation attempts and success and that current smokers will reduce their smoking. However, due to the multifaceted emotional stress caused by the pandemic, there is an increase in smoking in most countries during this period (Carreras et al., 2022).

At this point, it is seen that the COVID-19 pandemic has caused positive effects on some smokers and negative effects on others (Matsungo & Chopera, 2020; Pettigrew et al., 2020). More studies are needed to analyze nicotine dependence and smoking behavior in the COVID-19 pandemic. In this study, we will investigate the change in tobacco use behavior during the pandemic in a group of patients with COVID-19, a contact group and a group of healthy patients without any contact.

Smoking cessation is primarily a matter of intention. Therefore, participants should be asked about their smoking status and intentions. The risk of complications and mortality associated with smoking and COVID-19 infection is well known. Given this information, it is expected that smoking will be reduced and cessation will increase during the pandemic period.

The aim of this study was to investigate whether there is a change in the smoking behavior of individuals during the pandemic period. In addition, to determine the smoking and nicotine dependence status of COVID-19 positive, contacted or healthy individuals were compared to see if there was a change during the COVID-19 pandemic period.

Methods

Study Design

Our study is a cross-sectional, single-center study.

Ethical Approval

Prior to the study, study permission was obtained from the Ministry of Health with the application number 2021-09-13T10_07_01. Afterwards, ethical approval was obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (approval date: 30.09.2021/06).

Setting and Study Period

Our study was conducted in the Family Medicine Unit of Campus Education Family Health Center, which operates under Atatürk University Family Medicine Department, between October and December 2021.

Participants

In our study, simple random sampling was used, which was conducted on patients who presented to the Education Family Health Center with COVID-19 symptoms and complaints during the study period. Among these patients, patients over the age of 18 who were smokers, patients who were referred due to suspicion of COVID-19 and whose RT-PCR test result was positive, healthy people who were in isolation due to COVID-19 contact and who did not have any COVID-19 contact and symptoms were included in our study. Patients who were under 18 years of age and had not smoked before, pregnant, puerperant, emergency patients, patients with poor level of consciousness and patients who did not volunteer to participate in the study were not included in our study.

Patients who met the study criteria formed three groups. Among these patients, patients with COVID-19 symptoms and complaints and positive RT-PCR test results constituted the COVID-19 positive group, patients who were COVID-19 negative but were in isolation due to their contacts constituted the contact group, and healthy people without any COVID-19 contact and symptoms constituted the healthy group.

Instrument

In order to avoid contact with the patients included in our study and to prevent COVID-19 transmission and spread, they were asked to answer the study survey questions online or by phone. The demographic characteristics, smoking status, smoking status of the patients included in our study by fulfilling the inclusion criteria, smoking status before COVID-19 positive result or contact, and the change according to the COVID test result or after contact were evaluated with the Fagerström smoking dependence test.

The Fagerström test of smoking dependence is the most widely used tool for diagnosing smokers who want to quit and for assessing smoking dependence. It has been shown to predict smoking cessation outcomes (Svicher et al., 2018). The Turkish translation and validation study was conducted by Uysal et al. with a Cronbach alpha value of 0.56 (Uysal et al., 2004). The test can be self-administered or administered by an interviewer. It has six questions with different scores, and participants will score from 0 to 10, with higher scores indicating higher addiction, 0-3 scores indicating minimal addiction, 4-6 scores indicating moderate addiction, and 7-10 scores indicating high addiction (Heatherton et al., 1991).

Statistical Analysis

The data obtained and the differences between the groups were statistically investigated with the SPSS 23.0 program. Categorical data were presented as frequency and percentage, numerical data were presented as mean and standard deviation. Chi-square test was used in the statistical analysis of categorical data. Shapiro-Wilk test was used to determine the normal distribution of the data. One way ANOVA test was used in groups with normal distribution and Kruskal Wallis test was used in the analysis of independent groups in groups without normal distribution. Tukey test was used in post-hoc analysis and Spearman correlation test was used in correlation analysis. Statistical significance was taken as $p<0.05$.

Results

A total of 131 participants out of 167 patients were included in the study; the number of participants in the healthy group was 70, the number of participants in the COVID-19 negative but contacted group was 31, and the number of COVID-19 positive participants was 30. The proportion of male participants was 60% (n=42) in the healthy group, 83.9% (n=26) in the COVID-19 negative group, and 73.3% (n=22) in the COVID-19 positive group, with a borderline significant difference between the groups (p=0.048) (Table 1). The mean age was 41.09 ± 12.85 years in the healthy group, 38.21 ± 11.69 years in the COVID-19 negative group, and 39.47 ± 11.66 years in the COVID-19

positive group, and there was no significant difference between the groups (p=0.360). The proportion of participants with chronic diseases was 56.4%, 72.0%, and 72.0% in the healthy group, COVID-19 negative group, and COVID-19 positive group, respectively, and there was no statistical difference between them (p=0.250). Fagerström dependence scores were 1.82 ± 1.05; 1.53 ± 0.86; and 1.40 ± 0.72 in the healthy group, COVID-19 negative group, and COVID-19 positive group, respectively, with no statistical difference (p=0.175) (Table 2).

Table 1. Comparisons of Sex Distribution and Chronic Disease Existence in Study Groups

		Healthy		**COVID-19 Negative**		**COVID-19 Positive**		**Chi-square**	**P**
		Count	Column %	Count	Column %	Count	Column %		
Sex	Male	42	60.0%	26	83.9%	22	73.3%	6.082	**0.048**
	Female	28	40.0%	5	16.1%	8	26.7%		
Chronic Disease	No	31	56.4%	18	72.0%	18	72.0%	2.773	0.250
	Yes	24	43.6%	7	28.0%	7	28.0%		

(Note: Bold *P* value indicates statistical significance.)

The daily cigarette use of the participants before and after the pandemic is presented in Table 3 and it was found that the average cigarette use rates decreased in all three groups. Statistically, it was determined that there was a significant decrease in smoking rates in all three groups compared to before the pandemic (p<0.01). When the smoking status of the participants before and during the COVID-19 pandemic was questioned, it was found that there was a decrease in cigarette consumption of 1.13 ± 4.17 in the healthy group, 3.97 ± 5.31 in the COVID-19 negative group, and 10.14 ± 7.86 in the COVID-19 positive group, which was statistically significant (p<0.001) (Table 4).

When the participants were questioned about their desire to quit smoking, there was a statistical difference between the groups (p=0.045), but in post-hoc analysis, those in the COVID-19 positive group were more likely to consider quitting smoking. According to gender, although males and those without chronic diseases were more likely to consider quitting smoking, there was no statistical difference (p>0.05) (Table 5).

The Fagerström dependence score was 1.58 ± 0.87 and 1.75 ± 1.05 in those who considered quitting smoking and those who did not, respectively, but there was no statistical difference between them (p=0.330).

Table 2. Comparison of Age Distribution and Fagerstrom Nicotine Dependence Test Scores Among Study Groups

		Age	Chi-square	P	Fagerstrom	Chi-square	P
Healthy	Mean	41.09	0.838	0.360	1.82	1.837	0.175
	Std. Deviation	12.85			1.05		
	Median	42.50			1.50		
	Minimum	19.00			1.00		
	Maximum	68.00			5.00		
COVID-19 Negative	Mean	38.21			1.53		
	Std. Deviation	11.69			0.86		
	Median	36.00			1.00		
	Minimum	19.00			1.00		
	Maximum	59.00			4.00		
COVID-19 Positive	Mean	39.47			1.40		
	Std. Deviation	11.66			0.72		
	Median	39.50			1.00		
	Minimum	18.00			1.00		
	Maximum	59.00			4.00		

The correlation according to the age, Fagerström score, number of cigarettes use before and during the pandemic of the participants in the groups is presented in Table 6. Accordingly, the amount of smoking before the pandemic and smoking during the pandemic were positively correlated in all groups. In the healthy group, there was a weak positive correlation between age and smoking before and during the pandemic. In both the healthy group and the COVID-19 negative group, there was a strong positive correlation between the Fagerström score and the amount of smoking before and during the pandemic. In the COVID-19 positive group, there was a positive correlation between Fagerström score and smoking only before the pandemic.

Table 3. Comparison Between Study Groups of Participants' Previous and Current Amount of Smoking Per Day

		Previous Smoking (Count/Day)	Recent Smoking (Count/Day)	Z	P
Healthy	Mean	13.34	12.21	-2.668	**0.008**
	Std. Deviation	9.12	8.51		
	Median	10.00	10.00		
	Minimum	1.00	1.00		
	Maximum	40.00	40.00		
COVID-19 Negative	Mean	14.20	10.23	-3.523	**<0.001**
	Std. Deviation	9.06	8.98		
	Median	15.00	7.00		
	Minimum	2.00	0.00		
	Maximum	40.00	40.00		
COVID-19 Positive	Mean	13.54	3.39	-4.377	**<0.001**
	Std. Deviation	8.80	3.20		
	Median	10.00	3.00		
	Minimum	1.00	0.00		
	Maximum	30.00	10.00		
Chi-square		0.230	2.149		
P		0.631	0.143		

(**Note**: Bold *P* values indicate statistical significance.

Table 4. Daily Change in Past and Current Smoking of the Study Group

		Daily Smoking Amount Change	Chi-square	P
Healthy	Mean	1.13	52.389	**<0.001**
	Std. Deviation	4.17		
	Median	0.00		
	Minimum	0.00		
	Maximum	30.00		
COVID-19 Negative	Mean	3.97		
	Std. Deviation	5.31		
	Median	1.50		
	Minimum	0.00		
	Maximum	18.00		
COVID-19 Positive	Mean	10.14		
	Std. Deviation	7.86		
	Median	7.50		
	Minimum	0.00		
	Maximum	27.00		

(Note: Bold *P* value indicates statistical significance.)

Table 5. Comparison of Study Groups in Terms of Participants with and without Smoking Cessation Plans

		Planning to Quit Smoking		Not Planning to Quit Smoking		Chi-square	P
		Count	Row %	Count	Row %		
COVID- 19 Groups	Healthy	34_a	49.28%	35_a	50.72%	6.207	**0.045**
	COVID-19 Negative	15_a	48.39%	16_a	51.61%		
	COVID-19 Positive	7_a	23.33%	23_b	76.67%		
Age	1	34	38.20%	55	61.80%	2.735	0.098
	2	22	53.66%	19	46.34%		
Chronic Disease	No	26	38.81%	41	61.19%	1.241	0.265
	Yes	19	50.00%	19	50.00%		

(**Note:** Similar lowercase letters indicate statistical equality, while different lowercase letters indicate statistical difference. Bold P value indicates statistical significance.)

Table 6. Spearman's Correlation Test Results of Study Groups

			Age	Fagerstrom	Previous Smoking	Current Smoking
Healthy Group	**Age**	**r**	1.000	0.226	0.258*	0.283*
		p		0.063	0.034	0.019
	Fagerstrom	**r**		1.000	0.753**	0.707**
		p			0.000	0.000
	Previous Smoking	**r**			1.000	0.945**
		p				0.000
	Current Smoking	**r**				1.000
		p				
COVID-19 Negative Group	**Age**	**r**	1.000	0.275	0.318	0.232
		p		0.156	0.099	0.235
	Fagerstrom	**r**		1.000	0.751**	0.721**
		p			0.000	0.000
	Previous Smoking	**r**			1.000	0.759**
		p				0.000
	Current Smoking	**r**				1.000
		p				
COVID-19 Positive	**Age**	**r**	1.000	0.371*	0.198	-0.077
		p		0.043	0.311	0.698
	Fagerstrom	**r**		1.000	0.640**	0.214
		p			0.000	0.274
	Previous Smoking	**r**			1.000	0.474*
		p				0.011
	Current Smoking	**r**				1.000
		p				

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Discussion

Within the scope of our study, demographic characteristics, amount of cigarette use and smoking cessation thoughts were compared between the groups whose RT-PCR test results were negative or positive due to the fact that the individuals who smoked during the COVID-19 pandemic were healthy or had symptoms and were contacts. According to the data we obtained, there was a statistically significant decrease in the amount of cigarette use in all three groups compared to before the pandemic, but the highest decrease in the amount of cigarette consumption was found in COVID-19 positive patients. The thought of quitting smoking was again found to be significantly different in the COVID-19 positive group. However, there was no significant difference between the groups in terms of gender, presence of chronic disease or Fagerström dependence test score.

Since COVID-19 patients present with pulmonary symptoms and clinic such as shortness of breath, cough,

pneumonia and smoking among the patient groups at risk for COVID-19 (Reddy et al., 2021), it can be thought that there is an awareness in the society for smoking cessation and thus smoking rates have decreased. On the other hand, it is also possible that smoking may have increased due to fear of COVID-19, uncertainties about the future and psychological stress caused by COVID-19 in other studies in the literature (Rahman et al., 2020). However, what may cause the greatest confusion is the existence of studies showing that smoking is protective against COVID-19 (Tsigaris & Teixeira da Silva, 2020). Therefore, there is a need to investigate the smoking behaviors and addiction levels of individuals in the COVID-19 pandemic in order to develop smoking-related counseling and health policy. In this study, no significant difference was found between the Fagerström dependence test score and the intention to quit smoking. In this context, smoking counseling should be offered by all physicians to all groups of smokers, regardless of their addiction level.

The harmful effects of smoking on health and its association with lung diseases are well known (O'Keeffe et al., 2018; Strzelak et al., 2018). In the current COVID-19 pandemic, as smoking worsens symptoms and prognosis (R. Patanavanich & S. A. Glantz, 2020), more practices and counseling to encourage smoking cessation are needed. As found in our study, the population decreased their smoking rates and are tender to quit smoking, this tendency should be empowered and consultations should be applied.

Limitations

Our study has several limitations. First, the sample size is small and the study was conducted in a single center, which limits the generalizability of the study results. Multicenter studies with larger sample sizes are needed. Second, nicotine dependence and smoking cessation intentions were self-reported by participants and therefore may not be an accurate reflection of the truth. Third, the study was conducted in the middle of the pandemic, when everyone was still under the effects of COVID-19, which may have influenced the participants' decisions. Longer cohort studies are needed to see smoking cessation success rates.

Conclusion and Recommendation

It has been determined that smoking rates have decreased during the COVID-19 pandemic. Every individual who quits smoking with efforts to quit smoking will be an asset to society. In the COVID-19 pandemic, it should be an important goal for every physician to turn the decrease in smoking rates into an opportunity and to ensure smoking cessation by encouraging and strengthening smoking cessation counseling. With this goal, it should be planned to raise awareness, develop new strategies and new projects by investigating smoking and nicotine addiction levels.

Ethics Committee Approval: Ethical approval obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee.
Peer-review: Externally peer-reviewed.
Author Contributions: MD and MB conceived and designed the study. MD conducted the survey and collected the data. KK performed the statistical analyses. HAA drafted and wrote the paper. KK, MD, HAA, and MB discussed the results. MB supervised the study, interpreted the data, and finalized the paper.
Conflict of Interest: The authors have no conflicts of interest to declare.
Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Research Article DOI: 10.62425/rtpharma.1470761

Anticancer Potential of Watermelon Seed Extracts Against Lung and Breast Cancer Cell Lines

Fatma YEŞİLYURT[1]
Hilal ÖZBEK[2]
Mustafa Emir HACIMÜFTÜOĞLU[3]
Ayşe Neva COŞKUN[3]
Azranur DUMLU[3]
Osman COŞKUN[3]

[1] Department of Medical Pharmacology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye
[2] Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye
[3] Necmettin Erbakan Fen Lisesi, Erzurum, Türkiye

ABSTRACT

Objective: The aim of the study is to evaluate the anticancer potential of watermelon seed extracts against lung and breast cancer cell lines.

Methods: A549 lung cancer and MCF-7 breast cancer cell lines were used. The cells were treated with doses ranging from 0.1 to 1000 µg/mL of KI (inner part) and KD (outer part) extracts from watermelon seeds, starting when the cell density reached 80%. Viability was assessed using the MTT assay.

Results: For the A549 lung cell line, the KI extract demonstrated significant anticancer activity at doses of 10, 100, and 1000 µg/mL, with the 1000 µg/mL dose being the most effective against lung cancer cell line. Similarly, the KD extract showed efficacy across all doses tested, with the seed peel being effective at lower doses compared to the seed. In the MCF-7 breast cell line, both KI and KD extracts exhibited dose-dependent anticancer effects, with significant reductions in viability observed at all doses compared to the control group.

Conclusion: Interestingly, the seed and seed shell showed selective effectiveness against breast and lung cancer, indicating a dose-dependent and selective anticancer effect. Overall, these findings suggest the potential of watermelon seed extracts as promising anticancer agents with selective efficacy against different cancer types.

Keywords: Anticancer, A549, MCF-7, Watermelon

Received 25.03.2024
Accepted 02.05.2024
Publication Date 03.05.2024

Corresponding author:
Hilal Özbek
E-mail: ozbek@atauni.edu.tr
Cite this article: Yesilyurt, F., Özbek, H., Hacımüftüoğlu M.E., Coşkun, A.N., Dumlu, A., Coşkun, O. (2024). Anticancer Potential of Watermelon Seed Extracts Against Lung and Breast Cancer Cell Lines. *Recent Trends in Pharmacology, 2*(1), 21-26.

Introduction

Cancer is defined as one of the significant diseases worldwide with a high mortality rate (Aydın and Köseoğlu, 2022). It is a disease that can affect any organ or tissue in our body and arises from the transformation of our body's own cells for various reasons. The most significant characteristic of cancer is the abnormal cells gaining the ability to divide rapidly and uncontrollably, invading nearby tissues and organs without restraint, and furthermore, spreading to distant organs and tissues through the bloodstream. The most common cause of death from cancer is metastasis. While there are many known causes of cancer, there are also many unknowns about cancer today. About 30% of cancers can be prevented with simple measures. Many types of cancer, such as lung, breast, colorectal, laryngeal, and stomach cancer, can be treated when diagnosed early (Özyiğit, 2017). In addition, cancer patients are in search of many new treatment options in addition to the current ones.

Breast cancer is one of the most common cancers in women worldwide and represents a major public health concern. The MCF-7 cell line is a human breast cancer cell line commonly used in breast cancer research. This cell line is used to evaluate the effects of cancer drugs in in vitro studies. The MCF-7 cell line is a breast cancer cell line with estrogen receptors and is widely used in breast cancer studies (Aybek et al., 2020). The A549 cell line is a cell line derived from human lung adenocarcinoma and is widely used in lung cancer research (Xu et al., 2022). Genetic factors, environmental factors, dietary habits, sedentary lifestyle, infections, and exposure to chemicals can be listed among the causes of cancer.

Foods provide essential substances for the organism's metabolic needs, such as proteins, and additionally contain components, like secondary metabolites, that have positive effects on our health (Arı et al., 2017).

Healthy eating is crucial for preventing diseases. In particular, antioxidant nutrition is important to combat the effects of free radicals. Antioxidant nutrients can be defined as substances that can neutralize some or all of the adverse effects caused by free oxygen radicals that occur in physiological conditions in humans (Yılmaz, 2010). A good antioxidant eliminates free oxygen radicals in a specific manner, chelates redox metals, triggers other antioxidants within the antioxidant network, and has a positive effect on gene expression (Arı et al., 2017). Antioxidants such as anthocyanins, and phenolic compounds play an important role in reducing the function of free radicals. It is necessary to have certain foods containing these elements in our daily diet. Recent studies have shown evidence supporting the appropriate use of particularly red and purple pigmented foods as anticarcinogenic (Dyshlovoy and Honecker, 2020). Examples of such foods include eggplant, damson plums, purple grapes, blackberries, blueberries, elderberries, purple onions, purple rice, lavender, and watermelon, (Aydın and Köseoğlu, 2022).

Watermelon (Citrullus lanatus [Thunb.] Matsum. & Nakai) is an annual creeping plant belonging to the Cucurbitaceae family. The fleshy part of the mature fruit (endocarp) can vary in color from yellow to red and contains numerous black seeds. Watermelon seeds contain approximately 30-40% protein and 45% edible oil. This oil is rich in linoleic acid, oleic acid, palmitic acid, and stearic acid (Baser, 2022). The coumarins isolated from watermelon seeds have been reported to be effective against the abnormal growth of various cancer cell lines (Mustafa et al., 2024). Also it is known that antioxidant compounds such as lycopene and tocopherol found in extracts from watermelon fruit have biological effects on lung cancer (Di Sano et al., 2022). Phytol isolated from watermelon sprouts inhibited the growth of human T-cell leukemia Jurkat cells and suppressed tumor progression in human lung adenocarcinoma epithelial cell line (Itoh et al., 2018).

In this study, the effect of the extract obtained from watermelon seeds on lung and breast cancer cell lines has been investigated.

Methods

Plant Material

Watermelon was brought from Şanlıurfa in September 2022. The outer part (KD, 11.128 g) and inner part (KI, 9.634 g) of watermelon seeds were used as the plant material.

Extraction

The specified amounts of plant materials were powdered to an appropriate size and transferred to 500 ml erlenmeyer flasks. 100 ml of absolute ethanol (Sigma-Aldrich) was added to each flask. They were left for maceration overnight and extracted for 3 hours at 40°C on a magnetic stirrer. This process was repeated twice. After the extraction process, the extracts were filtered, combined, and the solvents were evaporated using a rotary evaporator at 40°C and 120 rpm. As a result, 96 mg of extract was obtained from the outer parts of the

watermelon seeds, while 75 mg of extract was obtained from the inner parts.

A549 Lung and MCF-7 Breast Cancer Cells Culture Experiments

A549 lung and MCF-7 breast cancer cell lines were obtained for our research from the Department of Medical Pharmacology, Atatürk University, Erzurum, Türkiye. Briefly, the cell suspension was centrifuged at 1200 revolutions per minute for 5 minutes. Cells were suspended in a new medium, Dulbeco's modified Eagle's medium (DMEM HG), containing 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin, streptomycin and amphotericin B). The cells were then collected in a flask with a surface area of 25 cm2. The flask was placed in an incubator with a carbon dioxide concentration of 5% and a temperature of 37°C. Once 80% of the flask is covered with cells, they will be extracted using a trypsin-ethylenediaminetetetraacetic acid (EDTA) solution (0.25% trypsin - 0.02% EDTA) followed by centrifugation. The liquid portion will be removed and the cell mixture will be dispensed into 96-well tissue culture plates at a volume of 100 µl per well (containing 10,000 cells per well).

Experimental Protocol

When the cells in the plates reached 80% density, the experimental design was established by determining 0.1, 1, 10, 100 and 1000 µg/ml concentrations for watermelon extracts. A wide range of concentrations was studied to find the effective dose. 96-well flasks were inoculated with pre-seeded cells reaching 80% density. Cells were cultured in 5% CO2 at 37°C for 24 hours. 10 replicates were used for each concentration.

MTT Assay (Cytotoxicity Analysis)

Foods provide essential substances for the organism's metabolic needs, such as proteins, and additionally contain components, like secondary metabolites, that have positive effects on our health (Arı et al., 2017).

The assay was completed after 24 hours, at which point 10 µl 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well. The samples were incubated for 4 hours after the addition of MTT. Dimethyl sulfoxide (DMSO) in a volume of 100 µl per well will be applied to dissolve formazan crystals formed after MTT treatment. A spectrophotometer (µQuant, Bad Friedrichshall, Biotek) was used to measure absorbance at a wavelength of 570 nm.

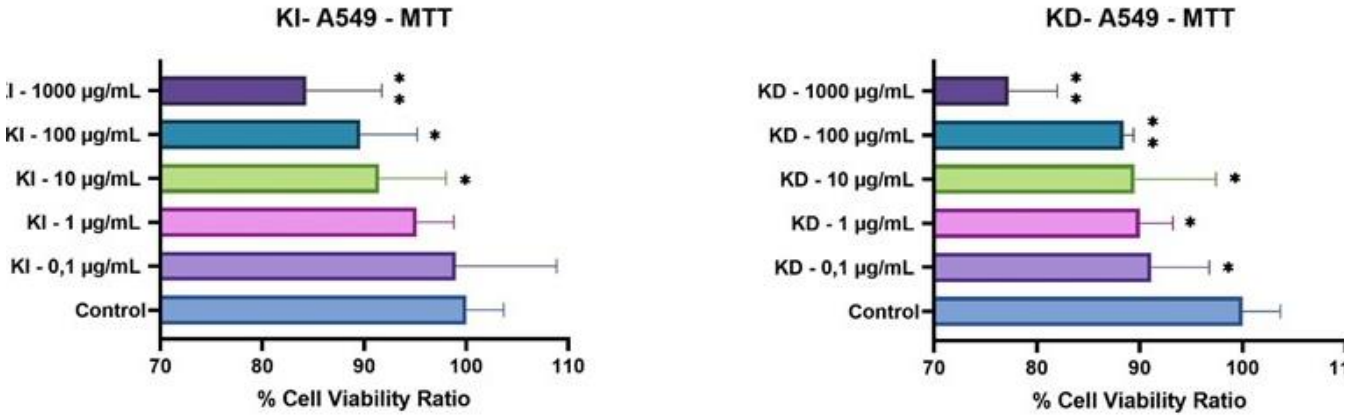

Figure 1. MTT results of KI and KD extracts in A549 lung cancer cell line (*p<0.05, **p<0.001 compared to control group)

Statistical Analysis

The data was analyzed using one-way analysis of variance (ANOVA) with post hoc Tukey's test (IBM SPSS 22.0). Statistical significance was determined at *p < 0.05 and **p < 0.001. The data were presented as the mean value plus or minus the standard deviation (SD).

Results

Experiments were performed with A549 lung cancer and MCF-7 breast cancer cell lines in cell culture. The experiment was initiated when the cell density in the wells reached 80%. Doses of 0.1, 1, 10, 100 and 1000 µg/mL of KI and KD extracts of watermelon seeds were prepared and A549 and MCF-7 cancer cell lines were treated for 24 hours. At the end of 24 hours, the cells were subjected to MTT assay and viability values were measured.

The MTT test results obtained by administering KI

extract to A549 lung cell line are presented in Figure 1. In the study, when the determined doses of the KI extract were compared with the control group, the viability rates in the 10, 100 and 1000 µg/mL groups were statistically significant. Cell viability levels were determined by proportioning the control groups to 100%. Watermelon seeds showed anti-cancer activity depending on the dose. 1000 µg/mL dose was found to be the most effective anticancer dose against lung adenocarcinoma type.

The MTT test results obtained by administering KD extract to A549 lung cell line are presented in Figure 1. all doses, the seed shell was found effective in lung cancer at all doses. In other words, there is a selective effect. At high doses, a killing rate approaching 25% in cancer cell numbers was obtained.

Discussion

Cancer is a pathological condition characterized by the uncontrolled proliferation and growth of cells in various organs or tissues of the body. Typically, cells undergo division and subsequent death in a specific order, but cancer cell lines deviate from this pattern (Ohshima and Morii, 2021). Cancer does not emerge only due to excessive and uncontrolled growth. In addition, the cell must exhibit additional malignant characteristics, including the ability to invade adjacent healthy tissues and migrate to neighboring healthy tissues via circulation, a process known as metastasis (Hanahan and Weinberg, 2000).

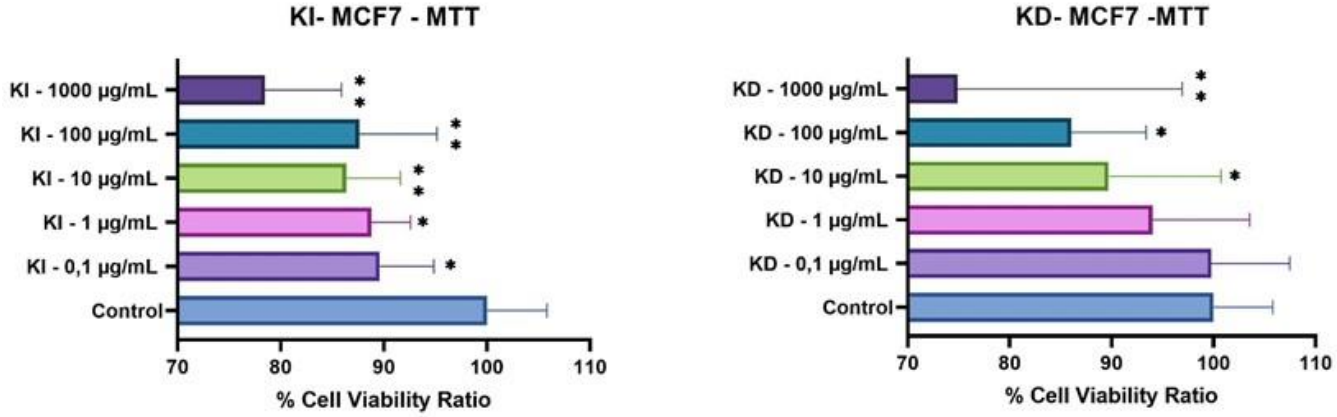

Figure 2. MTT results of KI and KD extracts in MCF-7 breast cancer cell line (*p<0.05, **p<0.001 compared to control group)

When the doses of KD extract were compared with the control group, the viability rates in all groups were found to be statistically different. It was an important result that watermelon seed peel was effective at the lowest 2 doses compared to the seed, while the effect could occur at the highest 3 doses tested in the seed. In addition, the dose-dependent increase in potency was similar for both watermelon seed extracts tested.

The MTT test results obtained by administering KI extract to MCF-7 breast cell line are presented in Figure 2. In the study, when the doses of the control group and KI extract were compared, the viability rates in all groups were statistically significant. The effect was dose dependent.

The MTT test results obtained by administering KD extract to MCF-7 cell line are presented in Figure 2. In the study, when the doses of KD extract were compared with the control group, the viability rates in the 10, 100 and 1000 µg/mL groups were statistically significant. Cancer killing rates were not significant at the lowest 2 doses.

While the seed was found effective in breast cancer at

The findings of this study demonstrate the potential anti-cancer properties of watermelon seed extracts, particularly the KI and KD extracts, against both A549 lung cancer and MCF-7 breast cancer cell lines. These results shed light on the dose-dependent efficacy and selective effects of the extracts, providing valuable insights into their mechanisms of action and potential applications in cancer treatment.

One of the key observations from this study is the significant decrease in cell viability rates observed in response to treatment with both KI and KD extracts across various doses. Particularly noteworthy is the substantial anti-cancer activity exhibited by the KI extract against A549 lung cancer cell lines, with statistically significant reductions in viability observed at doses of 10 µg/mL and above. This

indicates the potent cytotoxic effects of the KI extract on lung cancer cell lines, with the highest dose of 1000 µg/mL yielding the most pronounced anti-cancer activity.

Similarly, the KD extract also demonstrated notable efficacy against A549 lung cancer cell lines, with statistically significant reductions in viability observed across all doses tested. Interestingly, the KD extract showed effectiveness at lower doses compared to the KI extract, suggesting a potentially higher potency or different mechanism of action for the KD extract, possibly attributed to the presence of specific bioactive compounds concentrated in the seed peel.

In the case of MCF-7 breast cancer cell lines, both KI and KD extracts exhibited dose-dependent anticancer effects, with statistically significant reductions in viability observed across all doses tested. This indicates the broad-spectrum anticancer potential of watermelon seed extracts against different cancer cell lines, highlighting their versatility as potential therapeutic agents for breast cancer treatment.

Furthermore, the selective efficacy of watermelon seed extracts, with the seed shell demonstrating effectiveness against lung cancer cell lines and the seed against breast cancer cell lines, is particularly intriguing. This suggests the presence of differential molecular targets or cellular pathways in these cancer types that are selectively modulated by specific components of the extracts. Understanding the underlying mechanisms driving this selective effect could provide valuable insights into the development of targeted therapies for different cancer types.

Overall, the findings of this study underscore the promising anti-cancer properties of watermelon seed extracts and warrant further investigation into their therapeutic potential. Future studies focusing on elucidating the molecular mechanisms of action, identifying key bioactive compounds, and evaluating the efficacy of these extracts in in vivo models and clinical trials are warranted to fully harness their therapeutic benefits in cancer treatment.

Conclusion and Recommendations

This study demonstrates the selective and dose-dependent anticancer effects of watermelon seed extracts on A549 lung and MCF-7 breast cancer cell lines. Both KI and KD extracts exhibited dose-dependent efficacy against both lung and breast cancer cell lines. In conclusion, watermelon seed extracts hold promising potential as natural and effective anticancer agents. Further studies are needed to elucidate the mechanisms of action and in vivo efficacy.

Ethics Committee Approval: Since the cell line was studied in vitro, an ethics committee decision is not required.
Informed Consent: Since it is an in vitro study, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - FY, MEH, ANC, AD, OC; Design- FY, MEH, ANC, AD, OC; Supervision-OC; Resources- OC; Data Collection and/or Processing- OC; Analysis and/or Interpretation- FY, HÖ; Literature Search- FY, HÖ, MEH, ANC, AD; Writing Manuscript- FY, HÖ; Critical Review- FY, HÖ.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The study was supported by Tübitak 2204-A-High School Students Research Projects Competition 2023/1 (Project number: 1689B012338882).

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ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article DOI: 10.62425/rtpharma.1455410

In Silico Elucidation of the Binding Mechanisms and Molecular Dynamics of Oroxylin A -2,3-Dioxygenase Interaction: An Insight into Therapeutic Potentiation of Quercetin's Cardioprotection

Azizeh SHADIDIZAJI[1,2]
Kağan Tolga CİNİSLİ[1]
Mohamad WARDA[3,4]
Öznur ALTUNLU[5]
Sahar MEMARKASHANI[6]
Farideh GHALAMFARSA[7]
Abdullah MENZEK[8]
Dilanur ATEŞ[3]

[1]Vaccine Development Application and Research Center, Atatürk University, Erzurum, Türkiye
[2]Social Security Organization Treatment Management, Tabriz, Iran
[3]Department of Physiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Türkiye
[4]Department of Biochemistry, Faculty of Veterinary Medicine, Cairo University, Egypt
[5]Department of Pharmacology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye
[6]Department of Biology, Faculty of Sciences, Guilan University, Rasht, Iran
[7]Department of Medical Biotechnology, Shiraz University of Medical Sciences, Iran
[8]Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Türkiye

ABSTRACT

Objective: Elucidating the intricate interplay between enzymes and natural compounds is essential for designing therapeutic strategies.

Methods: This study employs advanced computational techniques to explore the binding mechanisms between quercetin 2,3-dioxygenase (QDO) and oroxylin A, revealing specific interaction patterns and key residues crucial to the formation of the QDO-oroxylin A complex.

Results: Molecular docking simulations revealed a favorable binding affinity (docking score: -5.6 kcal/mol) between Oroxylin A and the active site cavity of QDO, which was supported by Oroxylin A's specific orientation (Pose 3). Despite an observed root-mean-square deviation (RMSD) value of 2.776 indicating a moderate deviation between the docked pose and the reference structure, the formation of two hydrogen bonds with GLN 93 chain D underscores specific molecular interactions driving the binding process. This hydrogen bond formation suggested the presence of a stable and specific binding mode between Oroxylin A and QDO, likely influencing the functional dynamics of the enzyme, necessitating further refinement and validation of the docking model.

Conclusion: The ensuing deliberation on the implications of Oroxylin A include its potential as a modulator of QDO activity, emphasizing the importance of molecular-level insights in comprehending enzyme-compound interactions. Oroxylin A, a quercetin 2,3-dioxygenase inhibitor, was used in combination with other agents to prolong the biological impacts of quercetin, thereby amplifying its antioxidant and anti-inflammatory effects. This strategic approach exhibits promise in augmenting cardioprotective benefits, immune system support, and protection against diverse pathological conditions. Subsequent considerations of dosage, bioavailability, and healthcare professional consultation are imperative for judicious supplementation, particularly in individuals with prevailing health conditions or medications. This ongoing in silico study is dedicated to revealing the potential synergistic interactions of Oroxylin A, potentiating the long-term effects of quercetin and advancing our understanding of these intricacies.

Keywords: Quercetin, Quercetin 2,3-dioxygenase (QDO), Oroxylin A, Molecular docking, Antioxidant, Cardioprotection

Received 28.03.2024
Accepted 02.05.2024
Publication Date 03.05.2024

Corresponding author: Kağan Tolga CİNİSLİ
E-mail:kagantolgacinisli2525@gmail.com

Cite this article: Shadidizaji, A., Cinisli, KT., Warda, M., Altunlu, Ö., Memarkashani, S., Ghalamfarsa, F., Menzek, A., & Ateş, D. (2024). In Silico Elucidation of the Binding Mechanisms and Molecular Dynamics of Oroxylin A -2,3-Dioxygenase Interaction: An Insight into Therapeutic Potentiation of Quercetin's Cardioprotection. *Recent Trends in Pharmacology, 2*(1), 27-35.

Introduction

Cardiovascular diseases (CVDs) stand as prominent contributors to global mortality rates and necessitate the development of novel therapeutic interventions to combat this global health burden (Liu et al., 2021). Quercetin, a flavonoid abundantly found in various dietary sources, has garnered increased amounts of attention for its potential cardioprotective effects (Oboh et al., 2016). Quercetin, a polyphenolic compound abundant in various vegetables, fruits, and beverages such as red wine and tea, it exhibits a diverse array of pharmacological activities (Batiha et al., 2020). As a potent antioxidant, quercetin scavenges reactive oxygen species (ROS) furthermore, it provides protection against oxidative stress-induced damage in vascular endothelial cells and cardiomyocytes (Chang et al., 2021). Additionally, quercetin demonstrates anti-inflammatory properties by inhibiting proinflammatory cytokines and regulating intracellular signaling pathways implicated in the development of CVD (Hu et al., 2019). Additionally, quercetin has been shown to inhibit platelet aggregation, endothelial dysfunction, and smooth muscle cell proliferation, thus exerting antiatherogenic effects (Li and Zhang 2023). The pathophysiology of CVD is multifactorial and involves complex interactions between genetic, environmental, and lifestyle factors (Aggarwal et al., 2023). Quercetin targets several key mechanisms implicated in the progression and development of CVDs, comprising oxidative stress, endothelial dysfunction, dyslipidemia, inflammation, and thrombosis (Zhang et al., 2023). By enhancing nitric oxide synthase produced by endothelial cells (eNOS) activity and promoting the molecule known as nitric oxide (NO) production, quercetin improves endothelial function and vascular tone, thereby reducing the risk of hypertension and atherosclerosis (Yamagata 2023). Furthermore, quercetin attenuates lipid peroxidation, inhibits the expression of adhesion molecules, and suppresses The pathway of nuclear factor-kappa B (NF-κB), resulting in diminished vascular inflammatory response and plaque formation (Yan et al., 2023). Moreover, quercetin modulates lipid metabolism by regulating the activation of genes involved in lipid synthesis and breakdown, resulting in improved lipid profiles and a reduced risk of dyslipidemia-related CVDs (Papakyriakopoulou et al., 2022). Experimental studies using cell culture and animal models have provided compelling evidence supporting the cardioprotective effects of quercetin. These studies demonstrated that quercetin supplementation attenuates myocardial ischemia–reperfusion injury, preserves heart function, and decreases the size of tissue damage in animal models of heart attack (Papakyriakopoulou et al., 2022; Bartekova et al., 2010). Moreover, research has demonstrated that administering quercetin improves hypertension, improve endothelial function, and inhibit atherosclerotic lesion formation in various animal models of CVD (Patel et al., 2018). In clinical trials and meta-analyses involving human subjects, quercetin supplementation has been linked to enhancements in endothelial function, blood pressure, lipid profiles, and glycemic control, suggesting its potential therapeutic utility in CVD prevention and management (Yamagata and Yamori 2020). However,the effectiveness of quercetin therapy is constrained by its poor bioavailability and rapid metabolism, which are primarily mediated by enzymes such as quercetin 2,3-dioxygenase (Guo et al., 2022). Recent studies have identified oroxylin A, a natural flavone extracted from Scutellaria baicalensis Georgi, as a potential modulator of quercetin metabolism through interaction with quercetin 2,3-dioxygenase (Wang et al., 2014). Recent research has explored strategies to modulate quercetin metabolism and enhance its therapeutic potential. One such approach involves the use of oroxylin A, a natural flavone found in the plant Scutellaria baicalensis Georgi. Oroxylin A has been shown to interact with QDO, potentially influencing the degradation pathways of quercetin and altering its biological effects.

Despite the promising findings from preclinical and clinical studies, several challenges must be addressed to realize the full therapeutic potential of quercetin in CVD treatment. These challenges include issues related to bioavailability, formulation optimization, dose selection, and standardization of quercetin-containing products (Alizadeh and Ebrahimzadeh 2022; Khan et al., 2021). Moreover, the heterogeneity of study designs, patient populations, and outcome measures in clinical trials necessitates careful interpretation of the existing evidence and emphasizes the necessity for randomized controlled trials and well-designed with longer follow-up periods and larger sample sizes. Additionally, future investigation efforts should focus on elucidating the underlying mechanisms of quercetin's actions on specific CVD subtypes and identifying potential synergistic interactions with other cardiovascular medications.

In addition to performing a computer-based investigation, this study aimed to closely examine the intricate relationship between quercetin 2,3-dioxygenase and oroxylin A. The goal was to uncover crucial insights that can improve the effectiveness of quercetin and open the door to innovative therapeutic strategies for CVD treatment. By using computational methods, we aimed to thoroughly explore this interaction, shedding light on its

potential as a target for CVD drug intervention. This research not only investigated the molecular dynamics (MD) of the interaction between quercetin 2,3-dioxygenase and oroxylin A but also emphasized its broader implications for cardiovascular health, offering a hopeful avenue for the advancement of novel medications for cardiovascular disease therapy.

Materials and Methods

Ligand and protein preparation

The preparation of ligands and proteins for molecular docking commenced with the creation of PDBQT files, facilitated by UCSF Chimera software version 1.17.3 UCSF Chimera serves as a versatile tool for molecular visualization and analysis platform renowned for its advanced features, including structure visualization, trajectory analysis, and sequence alignment. While UCSF Chimera remains freely available for academic purposes, its successor, UCSF ChimeraX, provides expanded capabilities and is recommended for activities including structure preparation, acquiring 3D structures and docking (Pettersen et al., 2004). For docking a strategy that involved a flexible ligand and a rigid protein was implemented (Totrov and Abagyan 2008), wherein the receptor molecule maintained a rigid conformation while the ligand exhibited flexibility. Preparing the protein receptor included eliminating water molecules, consolidating nonpolar atoms, and assigning Kollman and Gasteiger charges (Mustafa et al., 2023).

The 3D structure of Oroxylin A (CID: 5320315) was retrieved from the PubChem database, which contains a comprehensive platform interconnecting substance, compound, and bioAssay database) (Kim et al., 2023). Subsequently, optimization and minimization of Oroxylin A were performed using the ChemBioDraw Ultra 14 suite, which is renowned for analyzing and drawing chemical structures (PerkinElmer 2023).

To visualize Oroxylin A in 3D, PyMOL software was used; PyMOL is known for its ability to generate high-quality molecular structures and facilitate molecular analysis, including structure visualization and simulation trajectory visualization (Figure 1A).

The X-ray crystallographic coordinates of QDO (PDB: 1JUH) were obtained from the RCSB database, which serves as a comprehensive archive of three-dimensional structural data for biological molecules (Figure 1.B). Finally, the preparation of docking and minimization structures for both ligands and receptors were accomplished using software UCSF Chimera version 1.17.3, enabling comprehensive preparation for subsequent molecular docking simulations.

Docking Protocol for Molecular Interactions

At the first,the CASTp online server was used for detecting active site of protein (Tian et al., 2016). Subsequently, the grid box dimensions were established as (20 × 20 × 20 Å), with the grid center set at coordinates (51.823, 12.609, 32.233) relative to the QDO using the software's configuration parameters. Molecular docking simulations of Oroxylin A and QDO were conducted utilizing the UCSF Chimera+Autodockvina32 software platform (Trott and Olson 2010). AutoDock Vina, a widely used open-source software designed specifically for molecular docking, was used for the computational prediction of the preferred orientation of Oroxylin A in relation to QDO to form a stable complex. This analysis encompassed evaluating hydrogen bond interactions and binding affinities, providing crucial insights into the molecular interactions between Oroxylin A and QDO within the active site.

Interaction between Quercetin 2,3-dioxygenase and the Oroxylin A complex

For analyzing proteins and ligand interactions, the Protein Ligand Interaction Profiler (PLIP) online server was performed (Adasme et al., 2021). This online server offers a free platform for examining protein–ligand interactions, offering valuable insights into binding energies, binding modes and interaction types. Widely employed in structural biology and drug discovery, the PLIP server facilitates the exploration of protein–ligand interactions crucial for designing novel drugs and therapies. Additionally, a 2D representation of the QDO and Oroxylin A complex was obtained from Discovery Studio Visualizer v21.1.0.20298 software ((BIOVIA, Dassault Systèmes, San Diego, CA, USA). The BIOVIA Discovery Studio Visualizer software represents a robust molecular visualization tool equipped with advanced features tailored for small molecule and protein data analysis. Its functionalities include creating high-fidelity 3D structures, facilitating comprehensive structure analysis, visualizing simulation trajectories and molecular docking. Users can leverage features such as distance measurements, angle determination, dihedral analysis, neighbor identification, and visualization of electrostatic surface potentials. In this study, we employed the BIOVIA Discovery Studio Visualizer to generate both 2D interaction visualizations and 3D representations of the QDO and

Oroxylin A complex, allowing for detailed examination and analysis of their molecular interactions.

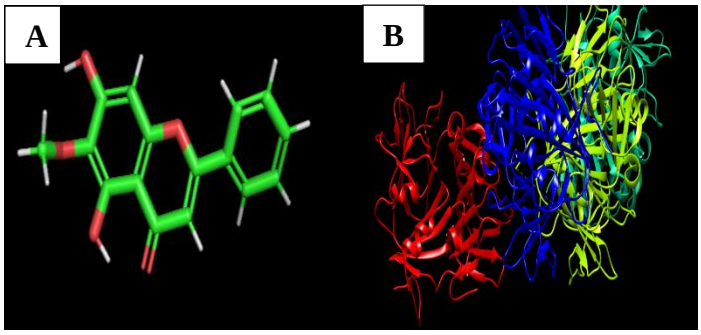

Figure 1. 3D structure of Oroxylin (A); Crystal structure of QDO (PDB: 1JUH) (B)

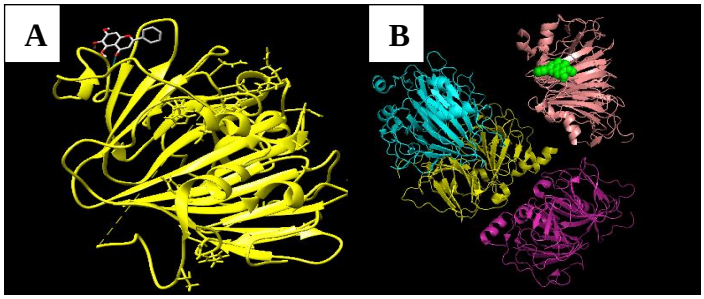

Figure 2. Docking of QDO and Oroxylin A (A); QDO and Oroxylin A complex (B)

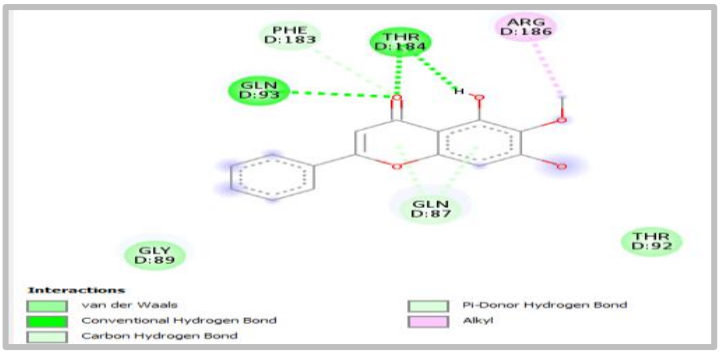

Figure 3. 2D diagram of the interaction between QDO and Oroxylin A

Table 1. Interaction between QDO and Oroxylin A according to the PLIP server

Hydrogen Bond									
Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein Donor	Side Chain	Donor Atoms	Acceptor Atoms
1	93D	GLN	2,57	3,14	110.07	V	V	8498 (Nam)	10355 (O3)
2	184D	THR	1.89	2.81	154.36	V	X	9079 (Nam)	10339 (O2)

MD Study

We utilized the GROMACS 2023 software (Rao et al., 2023) to conduct the simulations. To begin with, we prepared topology and coordinate files, employing the CHARMM36 force field for the intermolecular potential which was represented as a summation of Lennard-Jones (LJ) force and pairwise Coulomb interaction. The long-range electrostatic force was managed using the particle mesh Ewald (PME) method, and the numerical integration was executed through the velocity Verlet algorithm (Gharaghani et al., 2013). The system, under the condition of periodic boundary, was submerged in a water box shaped like a cube that contained water molecules with extended simple point-charge (SPC) atoms, positioned at a distance of 1 nm from each wall side. The neutrality of the system was confirmed, and 15 sodium ions were introduced in order to neutralize it, resulting in a system with 15 NA+ ions and 15756 solvent atoms. The energy minimization process, comprising of 50,000 steps for 2 fs, was followed by the equilibration stage at a constant temperature (NVT) of 300 K, utilizing the Berendsen thermostat, with a cutoff radius set at 1.2 nm. Subsequently, the system underwent equilibration at constant pressure (NVT) of 1 bar to optimize solvent molecules' arrangement around the solute. Finally, a 100 ns main simulation run at 300 K and 1 bar pressure was conducted.

To simulate the QDO-ligand complex, we utilized the SWISSPARAM server for the CHARMM36 force field to generate the topology file for the ligand. Subsequently, we integrated the topology parameters and ligand coordinates with those of QDO. The MD simulation of the protein-ligand complex mirrored the protein simulation and lasted for a duration of 100 ns. For all subsequent analyses associated with the simulation, such as the evaluation of intermolecular hydrogen bonds, RMSD, Rg, Root mean square fluctuation (RMSF) and solvent surface tension parameter (SASA) methods, we employed the GROMACS 2023 software. The MD simulation was carried out on an Ubuntu 22.04 Linux computer.

Results

The data provided here offer valuable insights into the molecular docking outcomes of Oroxylin A within the active site cavity TYDQAGSNCLEIPFVWH of QDO. A negative docking score of -5.6 kcal/mol indicated favorable binding affinity between Oroxylin A and QDO, while Pose 3 elucidated a specific orientation within the active site. Furthermore, the RMSD value of 2.776 revealed the deviation between the docked pose and the reference structure. Notably, Oroxylin A formed a hydrogen bond with GLN 93 chain D, suggesting its potential as a modulator of QDO activity. These findings underscore the promising pharmacological properties of Oroxylin A and its potential as a therapeutic agent in ongoing drug development endeavors (Figure 2A, B; Figure 3) (Table 1).

The initial pivotal stage in MD simulations involves assessing the RMSD, which signifies the stability and structural alterations occurring throughout the simulation period. RMSD represents the deviation of particle positions from the original structure at any given time (Sargsyan et al., 2017). A lower RMSD value indicates minimal fluctuation during the simulation, implying high stability of the protein (Elangovan et al., 2021). The mean average RMSD values for QDO and its complex with oryoxacilin were 0.325 and 0.239, respectively (Fig 4. D).

The RMSF algorithm evaluates regions of the structure with varying degrees of fluctuations compared to their reference structure. This parameter elucidates how ligand binding induces conformational changes at the residue level (Saravanan et al., 2019) (Fig. 4C).

The gyration radius is a measure used to assess compression changes during MD simulations. Protein compression during interactions with the ligand is influenced by protein chains and relies on the ligand's flexibility (Fig. 4E).

SASA values are employed to analyze the extent and significance of ligand binding to the receptor, as well as the alterations in protein conformation resulting from ligand binding (Shukla et al., 2019) (Fig. 4B).

Various interactions, including hydrogen bonds, hydrophobic interactions, and ionic interactions, stabilize the protein–ligand complex. Hydrogen bonds are particularly crucial and specific transient interactions for protein–ligand stabilization (Shukla et al., 2019; Eskandarzadeh et al., 2021) (Fig. 4A).

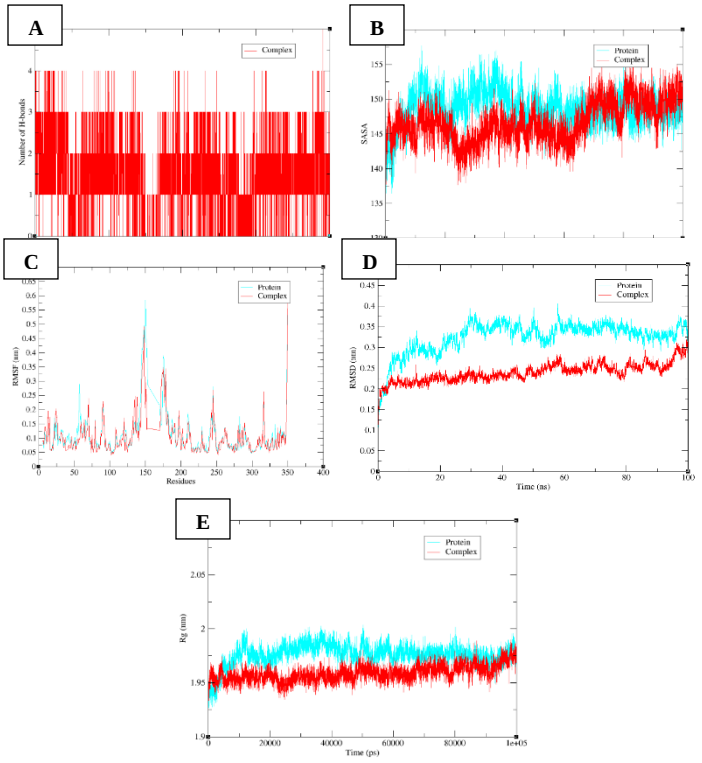

Figure 4. Molecular dynamics results generated by Gromacs software; number of hydrogen bonds ; SASA ; RMSF ; RMSD ; and Rg

Discussion

Quercetin, an extensively distributed natural compound found in diverse dietary sources, has attracted considerable attention owing to its purported therapeutic efficacy in the management of CVDs (Guillermo Gormaz et al., 2015). A growing body of research has elucidated its multifaceted properties, characterizing it as a promising candidate for promoting cardiovascular health. The documented antioxidant, anti-inflammatory, and antiatherogenic attributes of quercetin underscore its potential significance in mitigating the intricate pathophysiological processes associated with CVDs.

The antioxidant activity of quercetin is manifested through its ability to scavenge ROS, thereby providing a protective shield against oxidative stress, a pivotal player in the initiation and progression of cardiovascular malignancies. Moreover, the cardioprotective effects of quercetin are attributed to its anti -inflammatory properties by dampening the inflammatory cascade implicated in the development and exacerbation of cardiovascular pathologies. Its ability to modulate lipid metabolism adds another dimension to its therapeutic potential, as dysregulated lipid homeostasis is intricately

linked to atherosclerotic processes underlying CVDs. As scientific understanding advances, the exploration of the intricate molecular interactions of quercetin and its translation into targeted therapeutic interventions holds promise for addressing the complex landscape of cardiovascular health with precision and efficacy.

However, the therapeutic efficacy of quercetin is limited by its susceptibility to degradation by enzymes such as QDO, which can compromise its bioavailability and effectiveness (Siegbahn, 2004). This limitation underscores the importance of identifying inhibitors of QDO, such as oroxylin A, to preserve the beneficial effects of quercetin in managing CVDs.

Oroxylin A, a natural flavone compound, has shown promise as an inhibitor of QDO, thereby potentially enhancing the bioavailability and efficacy of quercetin in combating CVDs (Ren et al., 2020). By inhibiting the degradation of quercetin, Oroxylin A may prolong its therapeutic effects and improve its potency in cardiovascular health management.

In this comprehensive investigation, molecular docking outcomes were examined, and subsequent MD simulations were subsequently conducted to elucidate the intricate interaction between Oroxylin A and QDO. This molecular docking study provides insights into the underlying binding mechanisms involved, unveiling a favorable binding affinity with a computed docking score of -5.6 kcal/mol, indicating a thermodynamically favorable interaction at the active site cavity of QDO. This interaction is further supported by the specific orientation of Oroxylin A, as exemplified by Pose 3. Despite a moderate deviation with an observed RMSD value of 2.776 between the docked pose and the reference structure, the formation of two hydrogen bonds with the GLN 93 chain D underscores the specificity and stability of the QDO-Oroxylin A complex (Shadidizaji et al., 2024).

The results of the molecular docking analysis revealed a substantial negative docking score of -5.6 kcal/mol, indicating a robust and favorable binding affinity between Oroxylin A and QDO (Shadidizaji et al., 2023). The significance of this interaction is underscored by the specific orientation observed within the active site, emphasizing Oroxylin A's potential efficacy in modulating QDO activity (Shadidizaji et al., 2023). The establishment of a hydrogen bond with GLN 93 chain D further substantiates the specificity of Oroxylin A in its interaction with QDO, suggesting its potential role as a therapeutic agent (Shadidizaji et al., 2024; Rad et al., 2023).

Subsequent MD simulations were employed to determine the stability and structural dynamics of the QDO-Oroxylin A complex. Despite the moderate deviation revealed by the RMSD analysis between the docked pose and the reference structure, the mean average RMSD values indicate an enhanced stability of the complex compared to the unbound QDO (Matta 2003). Further analyses encompassing the RMSF, gyration radius, and SASA provided nuanced insights into conformational changes and protein–ligand interactions at the molecular level (Bayan et al., 2023; Rezaei et al., 2022).

The exploration of interactions stabilizing protein–ligand complexes has placed particular emphasis on hydrogen bonds as specific and transient interactions crucial for stabilization. This nuanced understanding of molecular interactions at the atomic level is pivotal for rational drug design and optimization, offering potential therapeutic avenues for oroxylin A in modulating QDO activity and addressing CVDs (Shadidizaji et al., 2023; Rad et al., 2023). These findings contribute to the broader landscape of structure-based drug discovery, providing a foundation for the development of targeted therapeutics with oroxylin A as a potential candidate for CVD intervention.

The results unveiled the pharmacological properties of Oroxylin A and its potential applications in therapeutic interventions, with a specific focus on CVD treatment. The study illuminates encouraging findings that underscore the inherent potential of Oroxylin A, positioning it as a compelling candidate meriting in-depth scrutiny within the realm of drug development initiatives directed toward ameliorating the complexities associated with CVDs and allied conditions. The identified outcomes not only elucidate the ability of Oroxylin A to interact with molecular targets implicated in cardiovascular pathophysiology but also emphasize its promising attributes, thereby suggesting its inclusion in the cohort of compounds earmarked for further exploration.

It is essential to acknowledge certain limitations in the current study. While computational approaches provide valuable insights into Oroxylin A and QDO interactions, rigorous experimental validation through in vivo studies and functional assays is necessary to confirm the modulatory effects of Oroxylin A on QDO activity and its impact on quercetin stability and functionality. Additionally, thorough exploration of the pharmacokinetics, bioavailability, and potential off-target effects of Oroxylin A is crucial for assessing its suitability as a therapeutic agent for CVD treatment. These

considerations emphasize the need for a comprehensive and multidisciplinary approach to clarify the potential of Oroxylin A as a QDO inhibitor, optimizing the effectiveness of quercetin for cardiovascular health.

Conclusion

In summary, the potential of quercetin for managing CVDs is hindered by enzymatic degradation, necessitating QDO inhibitors such as oroxylin A. Molecular docking and simulations reveal a strong binding affinity, suggesting that oroxylin A is a potential CVD intervention. Despite a moderate deviation, the QDO-Oroxylin A complex exhibited enhanced stability. Despite these limitations, further experimental validation and comprehensive exploration of the pharmacokinetics of Oroxylin A are crucial. This multidisciplinary approach is essential for optimizing the potential of Oroxylin A as a QDO inhibitor, as it enhances the effectiveness of quercetin for improving cardiovascular health.

Ethics Committee Approval: Ethical approval isn't necessary.
Peer-review: Externally peer-reviewed.
Author Contributions: KTC and AS designed the study. ÖA, MW collected the data. SM and FG drafted and wrote the paper. FG, AM, and DA discussed the results. MW supervised the study, interpreted the data and finalized the paper.
Conflict of Interest: The authors have no conflicts of interest to declare.
Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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ATATURK UNIVERSITY PUBLICATIONS

Review Article DOI: 10.62425/rtpharma.1458517

Pharmacological Treatment of Acute Spinal Cord Injuries in the Light of Recent Developments

Mürteza ÇAKIR
Department of Neurosurgery, Faculty of Medicine, Atatürk University Erzurum/Türkiye

ABSTRACT

Spinal injuries represent a significant public health issue with both individual and societal implications due to its potential to result in long-term or permanent disability and death. Today, notwithstanding the comprehensive elucidation of the mechanism of injury in its all aspects and breakthroughs in early diagnosis techniques and treatment, spinal injuries still retain their devastating nature. Although many agents hypothesized to possess neuroprotective and neuroregenerative properties have been demonstrated to be effective in the experiments, research involving human subjects is still in progress, offering promising developments. Methylprednisolone at a high dose is the most extensively investigated therapeutic for acute spinal injuries. Despite significant controversy, it remains a viable treatment option. It is anticipated that combining stem cell transplantation with multiple pharmacological agents will yield more favorable outcomes.

Keywords: Spinal injuries, Pharmacological agents, Stem cell transplantation

Received 26.03.2024
Accepted 17.04.2024
Publication Date 03.05.2024

Corresponding author:
Mürteza Çakır
E-mail: murtezacakir28@gmail.com
Cite this article: Çakır, M. (2024). Pharmacological Treatment of Acute Spinal Cord Injuries in The Light of Recent Developments. *Recent Trends in Pharmacology, 2*(1), 36-47.

Introduction

Spinal injuries are complex traumas that mostly occur resulting from mechanisms of blunt trauma, involving damages including fractures and dislocations of bone structures in the vertebral column, as well as lesions such as tearing and rupture of the soft tissue surrounding the column and/or damages to the spinal canal or the spinal cord within it (Wyndaele & Wyndaele, 2006). Spinal injuries represent a significant public health issue with both individual and societal implications due to its potential to result in permanent disability and death. Today, although the mechanism of injury has been elucidated in all aspects and breakthroughs in early diagnosis techniques and treatment, spinal injuries still retain their devastating nature. Given the vital role of the vertebral column and spinal cord, such injuries are likely to cause physical, psychological, social and economic problems, thereby significantly impacting both the injured individuals and their families and social surroundings (Devivo, 2012). Hence, it is crucial to initiate the treatment for spinal injuries as early stage as possible and to ensure the optimal management and treatment for patients (Wyndaele & Wyndaele, 2006; Karsy, & Hawryluk 2019).

Mechanism of Injury and Physiopathology

For decades, it has been understood that spinal cord trauma involves both primary and secondary injury mechanisms of biological processes. This mechanism, which was first described by Allen in 1911, recognises that primary injury is an inevitable result of energy transfer and the initial traumatic impact. Direct mechanisms including compression, contusion, laceration, as well as sudden increase in tension in the spinal vascular structure can give rise to this condition (Kwon et al., 2004; Fehlings et al., 2017). Such injury has the potential to result in a complete or partial anatomical lesion in the spinal cord. Secondary injury encompasses several mechanisms that initiate immediately after trauma and extend over weeks. In addition to the systemic response of the entire body to trauma, secondary injuries are observed as a result of the local, biochemical, histopathological, ion-mediated and/or oxidative cellular responses, and alterations in the spinal cord. These mechanisms are often complicated and interconnected and may result in delayed or secondary death of neuronal and glial support cells (Can et al., 2021; Yılmaz & Kaptanoğlu, 2015). Although it is theoretically inferred that pharmacological agents have the potential to target and restore these trauma-induced cascades, limited progress has been achieved so far in this regard (Yılmaz et al., 2015; Allan et al., 2015).

Initial Management of Acute Spinal Injuries

Until clinically and radiologically proven otherwise, it is acted upon the presumption that there is a spinal injury in cases of multiple and high-energy traumas. It is imperative to evaluate and transfer such patient by ensuring immobilization of the spine immediately following the incident. Also, during the primary evaluation process in emergency departments, care should be taken to ensure spine immobilization during interventions including examination and imaging as well as advanced airway provision (Fehlings et al., 2017). Clinical conditions such as hypotension and bradycardia (spinal shock findings) resulting from loss of control over vagal tone, and paralysis of respiratory muscles, should be identified and controlled early during a systematic physical examination that encompasses all systems. Considering that spinal shock symptoms may be confused with hemorrhagic shock in a traumatised patient, a differential diagnosis should be established and appropriate shock treatment should be initiated. Prompt initiation of fluid replacement and vasopressors should be initiated in the case of hemodynamic deterioration (O'Toole et al., 2019; Hadley et al. 2002). Once the patient should be stabilised in terms of respiration, circulation and neurology, and placed within a safety perimeter with continuous monitoring, they should be sent for imaging (Fehlings et al., 2017; Allan et al., 2015).

Conventional and Current Medical Treatment Approaches for Spinal Injuries

Currently, ongoing trials on the treatment for traumatic spinal cord injuries aim to prevent and restore secondary damage at the cellular level, as well as to mitigate the systemic secondary effects of trauma, including hypotension and hypoxia. The primary medical treatments applied for these purposes can be categorized under the headings of "neuroprotective therapies" preventing the progression of cord damage, including vasoactive medications to improve spinal perfusion, and "neuroregenerative therapies" aiming to restrore neuronal regeneration and myelination. The definitive treatment for these injuries will be achieved through advances in regenerative therapies, like cell transplantation, that seek to restore the damaged spinal cord.

1. Neuroprotective Therapies in Acute Spinal Injuries

Nowadays, there are ongoing trials for neuroprotective therapies aimed at treating numerous neurological

pathologies. The pharmacological agents used for this purpose are mostly well-established drugs that have been proven to be effective. It is also widely recognized that optimizing the timing of administration is crucial for achieving the highest level of effectiveness. While some agents are currently undergoing trial phase, the findings from both animal and human research show promising outcomes for the treatment of spinal trauma.

a- Regulation of haemodynamics and vasoactive pharmacotherapy: One could anticipate hypovolemia and hypovolemic shock as outcomes of trauma. Nevertheless, hypotension and hypoperfusion may manifest in cases of spinal injuries where sympathetic innervation loss occurs resulting from spinal damage in the absence of hypovolemia. This condition, which is particularly common in injuries above the level of the 6th thoracic vertebra, is referred to as spinal or neurogenic shock. The clinical differentiation between this shock and classical hypovolemic shock is based on the presence of bradycardia rather than tachycardia. The detrimental impact of hypotension on the damaged spinal cord, and preventing hypotension and maintaining blood pressure at targeted values reduces mortality and improves neurological outcomes are well-documented (Can et al., 2021). Guidelines emphasize the importance of maintaining the Mean Arterial Pressure (MAP) value high as avoiding hypotension (Hadley et al., 2013; Cozzens et al., 2013). Treating the hypoperfusion caused by spinal shock, will require more than just therapies that fill the vascular bed used in hypovolemic shock, often vasopressors will be needed to be used to increase blood pressure. The most preferred pharmacological agents for this purpose are Dopamine (1-10 mg/kg/min) and Norepinephrine (1-20 mg/min), which induce vasoconstriction with α- and β-agonist activities and increase cardiac activity. Dobutamine, Epinephrine and Phenylephrine can also be used (Streijger, et al., 2017; Ryken et al., 2013). A number of studies on how cord perfusion can be increased in spinal injuries have obtained data suggesting that the drainage of Cerebrospinal Fluid (CSF) increases perfusion. Further, data suggest that when CSF drainage and elevation of Mean Arterial Pressure (MAP) are performed together, intrathecal pressure increases by 5.45 mmHg, and this increase positively affects spinal cord perfusion by 24% (Streijger, et al., 2017; Jutzeler et.al., 2023; Can et al., 2021).

b- Corticosteroid Therapy: There are numerous studies on the use of corticosteroids in spinal trauma. The earliest views on this subject suggested that steroids were beneficial due their anti-inflammatory activity. Subsequent studies have shown that methylprednisolone has a free-radical scavenging effect. As mentioned earlier, disruption of membrane integrity by free radicals is one of the most important causes of secondary tissue damage. It has been claimed that high-dose of methylprednisolone increase medullary blood flow in the early stage of spinal cord injury, thereby improving perfusion, reducing excitotoxicity and neuronal phagocytosis mediated by immune mediators. Dexamethasone and other steroids have not shown any efficacy (Coutinho et al., 2011). The results of the International Acute Spinal Cord Injury Study-I (NASCIS-I), comparing the efficacy of low-dose methylprednisolone with high-dose methylprednisolone, emphasised that high-dose methylprednisolone did not result in significant neurological recovery but was closely associated with adverse outcomes such as wound infection, pulmonary embolism, gastrointestinal hemorrhage, sepsis and high mortality risk (Bracken et al., 1985). Similarly, in NASCIS-II, which compared high dose methylprednisolone and Naloxone (Opiad antagonist) with a placebo in the first 12 hours following trauma, no significant difference was found in neurological outcomes between the study groups. However, when the results of the subgroup consisting of patients treated with methylprednisolone within the first eight hours were examined, which was included in the study methodology, it was observed that although motor power recovery was quite significant in patients in this group, while complication rates such as wound site infection and pulmonary embolism were lower (Bracken et al., 1990). The latest study on this subject, NASCIS III, compared high-dose methylprednisolone with an antioxidant 21- aminosteroid (trilazad mesylate) within the first eight hours of trauma. This study, which also compared 24-hour infusions of both agents, found no difference between the triazilad mesylate and methylprednisolone groups. However, it was claimed that patients receiving a bolus dose of methylprednisolone after spinal injury also undergo a 48-hour infusion, their neurological outcomes at one-year was favourable. After the NASCIS III study, a 24-hour methylprednisolone infusion was recommended in patients receiving treatment within the first three hours after trauma, and a 48-hour methylprednisolone infusion was recommended in patients receiving treatment within three to eight hours (Bracken et al., 1997). Many researchers have conducted studies adopting the protocols of NASCIS II and III and reported that these protocols, especially those of NASCIS III, did not result in a significant increase in neurological recovery and led to many severe side effects, including secondary deaths. A revision was made to the methylprednisolone recommendations in the guidelines issued by the American Association of Neurological Surgeons/Central Nervous System (AANS/CNS) approximately 15 years after the last NASCIS

protocol. It was underscored that the use of methylprednisolone in acute spinal injury lacked approval from the Food and Drug Administration (FDA), that there were no supporting findings of classes 1 and 2 for the clinical benefits of this administration, and that high-dose corticosteroid administration was associated with multiple complications, including death, as indicated by findings of classes 1, 2 and 3. Consequently, it was highlighted that it is more appropriate to administer high-dose methylprednisolone for 48 hours rather than 24 hours, and treatment should commence within the first eight hours rather than eight hours following the injury (Hurlbert et al., 2015). In contrast, a large meta-analysis conducted in 2020 stated that methylprednisolone treatment within the first 8 hours did not yield a statistically significant short- or long-term improvement in overall motor or neurological scores of patients compared with steroid-free controls. Furthermore, it induced an increased risk of pneumonia and hyperglycaemia compared to controls, indicating that its routine use should be carefully considered. The use of steroids for acute spinal cord injuries and if used, the strategy to be followed, remains controversial today.

c- Minocycline: Minocycline is actually a synthetic, antibiotic of tetracycline class that has been tested in oncological and degenerative diseases of the nervous system, Alzheimer's disease and stroke. In recent years, it has also been used in acute spinal injuries. It plays a neuroprotective role through its multifaceted mechanism of action with its anti- inflammatory, antioxidant and apoptosis inhibitory properties. Many preclinical studies have shown that it improves motor functions, reduces lesion size and provides axonal protection (Festoff et al., 2006; Wells et al., 2003). A Phase II study on minocycline emphasises that although it is not very effective in lumbar spinal injuries, there are positive data on motor recovery in cervical spinal injuries (Casha et al., 2012).

d- Ganglioside GM-1: A glycolipid molecule located in the membranes of mammalian central nervous system cells has been indicated to exhibit potential neuroprotective effects in acute spinal injuries by experimental studies. In addition to its anti-apoptotic and excitotoxicity-inhibiting effects, it also accelerates axonal regeneration (Can et al., 2021). Although the results from initial studies on the use of this molecule for acute spinal injuries are promising, similar results have not yet been obtained from more comprehensive, multicentre and long-term patient follow-up studies. Therefore, it is not included in the new guidelines (Cozzens et al., 2013; Jutzeler et al., 2023).

e- Riluzole: This agent, a sodium channel blocker and an anticonvulsant of the benzothiol class, has been used for the treatment of Amyotrophic Lateral Sclerosis since the discovery of its neuroprotective effects in the 1990's. It inhibits glutamate excretion presynaptically and mediates glutamate transfer in synaptic intervals. It inhibits guanylyl cyclase cascade by voltage-dependent sodium channel blockade and limits the excitotoxic effects of glutamic acid released by cellular death. It is the only drug approved for neuroprotective activity. There are experimental studies and completed phase trials on the use of Riluzole in acute spinal injuries (Can et al., 2017).

f- Amantadine: Although primarily an antiviral medication, this agent is also used in the treatment of Parkinson's disease. It is believed to be effective by inhibiting the reuptake of dopamine in the synaptic cleft and increasing dopamine release from vesicles, thus showing high dopaminergic activity. Additionally, it acts as a sympathomimetic. The survival-enhancing and neuroprotective properties of this agent are thought to be exerted through dopaminergic, sympathomimetic, and N-methyl-D-aspartate receptor antagonism (Yılmaz & Kaptanoğlu, 2015).

g- Glyburide: Glyburide, also known as glibenclamide, is used as an antidiabetic agent. It is a nonspecific cation channel blocker and regulator of sulfonylurea receptor-1. In addition to stimulating insulin release, it is claimed to reduce hemorrhagic necrosis, oedema and inflammation through its effect in the microvascular area, thus leading to successful results in experimental modelling of hemorrhagic stroke and traumatic brain injury. It has been found that decreases in bleeding up to 24 hours following injury or bleeding, and the lesion begins to shrink within six weeks (Kurland et al., 2013; Popovich et al., 2012).

h- Magnesium (Mg): This element, which is a factor in the healthy functioning of many systems in the human body, has been found useful as a neuroprotective agent in many central nervous system diseases, including cerebral palsy. Mg, which is an N-methyl- D-aspartate receptor antagonist, reduces inflammation by inhibiting cytokines and reduces free radical levels. It prevents glutamate-dependent excitotoxicity. In two experimental studies on the efficacy of Mg on traumatic spinal injury, Mg was administered in polyethylene glycol, which facilitates blood brain barrier passage and improves biodistribution, and was found to be more effective than methylprednisolone, especially in the return of motor functions (Kwon et al., 2009; Lee et al., 2010). However, the effectiveness of Mg in spinal injuries has not yet been proven in human studies (Temkin et al., 2007). Phase II human studies on Mg are still

ongoing today (Karsy et al., 2019).

i- Granulocyte Colony Stimulating Factor (G-CSF): A glycoprotein produced endogenously which induces the production and release of granulocyte and stem cells into circulation by stimulating the bone marrow. It is claimed to promote functional recovery and provide neuroprotection in many degenerative nervous system diseases. It has been emphasised that non-hematopoietic functions such as protection of myelin structure, stimulation of angiogenesis, and TNF-□ and IL-1 suppression also contribute to this effect (Karsy et al., 2019). However, in two separate studies conducted on patient groups with spinal injury, although ASIA scores improved in the follow-up of patients treated with autologous stem cells and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), no improvement in neurological functions or reduction in toxicity reduction were detected after treatment (Park et al., 2005; Yoon et al., 2007).

j- Naloxone: This agent, an opiate antagonist, is thought to be effective in acute spinal injuries as it decreases the activity of nitric oxide synthetase and superoxide dismutase. In NASCIS-II, it was shown that there was no difference between methylprednisolone and placebo treatment groups in terms of motor recovery. (Bracken et al., 1990).

k- Erythropoietin: It is known that this molecule exerts its non-hematopoietic glioprotective and neuroprotective effects by reducing medullary cavitation, cell infiltration and apoptosis. Its derivatives produced via recombinant technology that do not induce erythropoiesis, yet to be tested in human trials, are considered promising for spinal traumas (Alibai et al., 2015). Furthermore, new studies have been conducted to investigate the combination of erythropoietin with more established pharmacotherapies for the treatment of traumatic spinal injury (Ganjeifar et.al., 2021).

l- Rolipram: While clinical trials have yet to establish its effectiveness, experimental evidence suggests that Rolipram can improve motor and sensory functions in traumatic spinal injuries in rats. The anti-inflammatory effects of Rolipram, a phosphodiesterase inhibitor, are thought to be responsible for these outcomes (Nikulina et al., 2004).

m- Nimodipine: A L-type calcium channel blocker that inhibits apoptotic enzymes and reduces the release of glutamate at synapses. It is known to regulate microvascular circulation, thereby increasing spinal cord blood flow. Although the first study on nimodipine was conducted in 1996, its efficacy on neurological recovery has not been proven superior to placebo (Fehlings et al., 2017).

n- Tirilazad mesylate: A synthetic 21-Amino-steroid molecule specially produced to inhibit peroxidation of lipids in neuronal membranes, was suggested that it has comparable efficacy to methylprednisolone in the NASCIS-III study. However, the lack of placebo-controlled studies and its similarity to methylprednisolone in terms of complications have reduced the availability of this drug (Bracken et al., 1997; Boyalı et al., 2020).

o- Mannitol: It is known that mannitol should be initiated for anti-edema treatment in spinal injuries at early stage where there are no contraindications without any reason (Huang et al., 2019).

p- Induced Hypothermia: In recent years, there has been considerable interest in the application of local or systemic induction of hypothermia for both the treatment of injuries and the care of comatose patients, due to its ability to reduce oxygen consumption by decreasing metabolic rate. Although there is not abundant supporting evidence, direct cooling of the spinal tissue intraoperatively has been used to treat spinal cord injuries for decades. Induced hypothermia was once more a subject of discussion in 2007, when it was nearly abandoned, after it was administered to an injured professional footballer and the patient regained sufficient motor function to walk within a very short time. Whether the early neurological recovery in this case was due to hypothermia is open to speculation. This favourable outcome may be due to early decompression and spontaneous neurological recovery, which have been observed in some cases (Kwon et al., 2008; Dietrich et al., 2011). There are no universally acknowledged indications or contraindications for induced hypothermic therapy. It is advisable to administrate it when there are no inhibitory factors associated with the patient and when adequate medical facilities are available (Martirosyan et al., 2017; Boyalı et al., 2020). Experimental research emphasizes that optimal temperature range for systemic hypothermia is 32-340C, which is considered moderate hypothermia (Ahmad et al., 2014). Local hypothermia can be administered via irrigating the epidural and/or subdural space with cold water at 6°C, inducing hypothermia (Dididze et al.,2013; Hansebout et al.,2014).

2. Neuroregenerative Therapies in Acute Spinal Injuries

Strategies targeting neural regeneration for treatment

of spinal injuries aim not to stop secondary injury, but rather to activate and/or strengthen the organism's own repair mechanisms. The main goal of these treatment strategies is to overcome factors that impede recovery such as inhibitory molecule signalling, scarring, loss of structural framework, cavitation. The correct timing of these treatment plans may vary depending on the patient, the general medical condition of the patient, as well as factors such as which strategy is more appropriate in which period. For example, some treatment plans are more beneficial in the acute phase immediately after the injury, while others are more beneficial in the subacute or chronic phase. The other concomitant therapies and the timing of these therapies are also of special importance (Ahuja et. al., 2016; Boyalı et al., 2020). Some of the most well-known neuroregenerative treatments are given below.

A- Myelin-linked inhibitor targeting therapy:

a. Anti-Nogo-A Antibodies: Based on the idea that Nogo-A, a proteinaceous building block of myelin, has a significant reducing effect on neuronal growth (Chen et al., 2000), experimental studies carried out by intrathecal injection of selective Nogo-A antibodies to some experimental animals, revealed that this antibody increased the restructuring and regeneration of axons in the damaged medulla spinal cord. Phase I and phase II clinical trials on this subject are ongoing (Zorner et al., 2010; Boyalı et al., 2020).

b. VX-210 (Cethrin®): A modified form of C3 transferase derived from C. botulinum with promising developments in its use for traumatic spinal injuries. The paste form of this therapeutic is known by the trade name Cethrin and can be administered directly to the dura mater during the operation. It is effective in axonal growth and functional recovery and has the ability to prevent apoptosis. It exerts this effect by inactivating Rho and Rho-Associated Kinase, which hinder neuronal growth (Forgione et al., 2014). Phase I/IIa trials on this topic have shown its effectiveness on motor recovery in injuries of the cervical and thoracic regions, more prominently in thoracic injuries. There was also an increase in sensory recovery in patients with thoracic injuries (Fehlings et al., 2011).

B- Treatment with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs):

Many types of this group of drugs, which are frequently used in clinical practice, contribute to axonal regeneration by inhibiting the Rho pathway. Experimental studies have shown that NSAIDs may target cyclooxygenases in spinal injuries and improve motor functions (Xing et al., 2011; Sharp et al., 2013).

C- Fibroblast growth factor (Fibroblast growth factor; FGF):

FGFs, which are potent mitogens that stimulate cell proliferation and regeneration of stem cells, are actually a collection of 22 proteins that signal with different tyrosine receptor kinases together with their own receptors. By increasing the proliferation of stem cells, it has been predicted that they may be included in treatment combinations in the treatment of traumatic spinal injury. In an experimental study, it was claimed that it may also be effective by promoting angiogenesis after spinal injuries (De Laporte et al., 2011). There is an FGF analogue with proven neuroprotective and neuroregenerative properties, but phase II trials have not been completed and no results regarding its effects in humans have been reported (Shi et al., 2014). Phase I trials for another FGF-impregnated biomedical device are undergoing (Karsy et al.2017).

D- Hepatocyte Growth Factor (HGF):

This molecule, known to act as a neurotrophic factor by stimulating angiogenesis, has been reported to be promising as it has been shown to protect fibres of the corticospinal tract in primate models of cervical spinal injuries and to be supportive motor functions of the upper extremities (Kitamura et al., 2011). Phase I/II studies of human-derived HGF obtained with recombinant technology are ongoing (Boyalı et al., 2020).

E- Chondroitinase ABC:

Another method that is thought to provide neuroregeneration in spinal injuries is the targeting of the existing glial scar tissue. Glial scar is a formation that impedes neuronal growth and the penetration of regeneration therapies. An experimental animal study demonstrated that chondroitinase ABC, an enzyme produced by bacteria, cleaves the glucose chains and proteoglycans in the glial scar and thus supports functional recovery (Bradbury et al., 2002). For the future medical treatment of spinal injuries, it has been suggested that the combination of this molecule with Anti-Nogo-A will have highly effective in providing therapeutic benefits (Zhao et al., 2013).

3. Cell Transplantation Approaches in Acute Spinal Injuries

Recent advancements in stem cell transplantation have

sparked new hypothesis regarding its potential therapeutic applications in acute spinal injuries. Prior to the current understanding of stem cells, it was postulated that central nervous system tissue lacked the capacity of regeneration. Nevertheless, this contention has been refuted by the demonstration that multipotent neuronal stem cells can differentiate into neurons, astrocytes and oligodendrocytes under favourable conditions (Barnabé-Heider et al., 2018). The strategy of stem cell transplantation in acute spinal injuries includes certain goals such as replacement of damaged neurons, stimulation the release of various trophic factors, and regulation of the microenvironment (Antonic et al., 2013). To date, however, only a handful of small studies have examined the efficacy of stem cell transplantation in patients with spinal trauma, furthermore, the results are highly variable (Donelly et al., 2012; Boyalı et al., 2020). Concerns regarding the risk of neoplasms arising from transplanted stem cells and limited functional recovery have not been ruled out in the studies conducted to date. Continuation of the treatment strategy with adjuvant therapeutics and combinations with one or more neurotrophic agents mentioned above are thought to improve the results (Boyalı et al., 2020; Taylor et al., 2006).

4. Neuropathic Pain Management in Acute Spinal Injuries

Gabapentin (GBP) and Pregabalin (PGB): Secondary clinical conditions following spinal injuries include depression, anxiety, sleep disorders and neuropathic pain, which are difficult to resolve. More than half of the cases following this form of injury exhibit neuropathic pain (Gustorff et al, 2008). It may cause the patient's daily activities, routine and quality of life to be disrupted, thereby exacerbating preexisting conditions. While anticonvulsants have been used for the treatment of this condition for years, today, GBP and PGB are the first-line treatment options for neuropathic pain caused by spinal injuries. PGB, a new generation of gabapentinoids with a comparable mechanism of action to GPB, is the only drug approved for this indication by the US Food and Drug Administration. For years, both GBP and PGB have been safety used as therapeutics for clinical conditions such as postherpetic neuralgia and diabetic peripheral neuropathy (Teasell et al., 2010).

5. Management of Complications in Acute Spinal Injuries

a- Management of respiratory complications: Clinical pathologies of the respiratory system such as recurrent pneumonia, atelectasis, and pleural effusion are common following spinal injuries. Pulmonary problems are particularly common in cases of upper or mid-level cervical spinal injuries, as damage to the phrenic nerve exit area. Symptoms such as dyspnoea, chest pain and cough may be present. Death due to pulmonary pathologies is common in cervical spinal injury. Treatment may involve frequent and deep breathing exercises, respiratory physiotherapy, and bronchial clearance if necessary. Mechanical ventilation is started if necessary with close blood gas monitoring. The patient should be followed up in appropriate wards or intensive care unit according to the patient's clinic status. Pharmacotherapy may include the use of agent-specific antibiotics, symptomatic treatment and bronchodilators (Hadley et al., 2002; Boyalı et al., 2020).

b- Management of cardiovascular complications: In the short period following cervical spinal injuries, activation of the sympathetic system is suppressed and parasympathetic dominance begins. Symptoms such as hypotension, bradycardic rhythm and increased secretions are common. We have mentioned that especially cervical and upper thoracic injuries can cause "neurogenic shock" and its management. In the absence of neurogenic shock, it will be appropriate to monitor the fluid balance to correct hypotension and to replace it when necessary. A vasopressor agent may be necessary to ensure adequate perfusion. Atropine (0.5-1 mg Iv push) can be used to correct symptomatic bradycardia (Karsy et al., 2019).

c- Management and prophylaxis of deep vein thrombosis (DVT): Patients with spinal injury are predisposed to DVT due to prolonged immobilisation. Clinically symptomatic DVT has been reported to be as high as 17%, and the incidence of DVT detected by imaging has been reported to be almost 80%. Consequently, the incidence of thromboembolic events such as pulmonary embolism is also increased. The risk is further increased in cases where spinal injuries are accompanied by pelvic and lower extremity fractures. Regular use of compression stockings and limb exercise are routinely recommended for the management of DVT risk in spinal injuries. Unless there are absolute contraindications, one of the prophylaxis regimens of low dose subcutaneous Heparin (5000 U) twice daily or Low Molecular Weight Heparin (Enoxaparin) 20-40 mg/day should be initiated. The primary goal should be to mobilise the patient as soon as the vertebral column is stabilized (Karsy et al., 2019; Can et al., 2021)

d- Management of gastroentrological complications and nutrition: Partial ileus may develop in most cases with spinal injury. In addition, caution should be exercised in such a patient, as the acute abdomen may progress without

clear clinical features. During the acute phase of the injury, a gastric tube should be inserted via nasal or oral route in order to prevent gastric distension and potential perforation. Sucralfate and proton pump inhibitors or H2 receptor antagonists can be employed for prophylaxis of acute gastroesophageal reflux disease and peptic ulcer which may result from both the stress caused by trauma and high dose steroid use. In patients with spinal injury, inadequate nutrition may lead to problems related to immunity and wound healing by causing catabolic energy supply. However, oral/enteral nutrition may not always be possible, particularly during the acute phase. In these cases, intravenous hyperalimentation rich in lipids should be initiated early. Enteral nutrition via jejunostomy or gastrostomy may be considered in indicated cases (Fehlings et al., 2017; Can et al, 2021).

e- Management of urological complications: Persistent urinary tract infections appear to be the most prevalent urological complication among patients with acute spinal injury. To prevent these infections from leading to hydronephrosis and renal failure, it is crucial to perform periodic bladder irrigation and urinary catheter use, as well as replacing the catheter at least once a week. In case of urinary infection, it should be treated with agent-specific antibiotherapy (Landi, 2003).

f- Management of hyponatraemia: The drop in serum sodium concentration that occurs approximately 6-9 days following trauma, reaches lowest level between 9-17 days, and typically rebounds to normal levels within 24-36 days. Despite the fact that clinical trials have documented varying frequencies ranging from 45% to 100%, it is evident that this is a commonly encountered complication. The main causes include high levels of cervical injury, concomitant infective conditions, intensive care conditions and ventilator use, and certain medications, especially diuretics. If the underlying cause is the Syndrome of Inappropriate Antidiuretic Hormone (SIADH), refined carbamide can be used, while Cerebral Salt Wasting Syndrome (CSWS), fludrocortisone can be used (Ohbe et al., 2019, Chavasiri et al., 2022).

g- Management of musculoskeletal and cutaneous complications: The most common skin and soft tissue issue encountered is pressure sores. Preventing the development of pressure ulcers is the most effective treatment strategy. This primarily entails ensuring the patient is properly positioned and repositioned frequently as well as using specialized manufactured pressure-relieving mattresses. Massaging the skin with moisturizing lotions can be beneficial. The skin should be carefully examined frequently and even superficial ulcers should be treated by covering with sterile occlusive dressings. Severe pressure sores may require surgical debridement. Common musculoskeletal problems such as contusions and spasticity can be minimised with physiotherapy (Eli et al., 2017).

Future Prospects in Medical Treatment of Spinal Injuries

Future prospects for the treatment of these types of injuries lie in more preventive or restorative strategies for secondary injury mechanisms. In the light of the studies conducted so far, it can be asserted that promising developments for favourable outcomes lie in combinations of numerous pharmacotherapies related to neuroprotective, neuroregenerative and stem cell transplantation therapies, whether their efficacy has been proven or they are still under investigation. Although the abundance of these combination options complicates the situation, the multiplicity of possibilities raises expectations for the future (Yılmaz et al., 2015; Fehlins et al., 2017; Can et al., 2021; Karsy et al., 2019).

Conclusion

Spinal injuries remains a formidable subject for scientific investigations owing to their complex pathophysiology, heterogeneity of patients and mechanism of injury, and serious comorbid conditions. Despite all the achievements, complete neurological recovery has not yet been achieved. The insights and expertise gained from the results of studies conducted thus far will assist in the development of future clinical management strategies. Expectations are high that more encouraging results can be achieved with multiple pharmacological agents accompanying stem cell transplantation.

Ethics Committee Approval: Since our study is a review article, ethics committee approval is not required.
Informed Consent: Since it is a review article, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - MÇ; Design- MÇ; Supervision- MÇ; Resources- MÇ; Data Collection and/or Processing- MÇ; Analysis and/or Interpretation- MÇ; Literature Search- MÇ; Writing Manuscript- MÇ; Critical Review- MÇ; Other- MÇ
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

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