https://doi.org/ 10.33472/AFJBS.6.9.2024.529-542

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

NEUROPROTECTIVE AND ANTIOXIDANT APPRAISAL OF MICROWAVE ASSISTED EXTRACT OF *NERIUM OLEANDER* LEAVES

Alambaram Vaishnavi[1], Anamika kulshrestha[2], Dighe Rajendra Dnyandeo[3*], Lakshmi[4], Soniya R[5], Dheeraj Upadhyay[6], A H V Santhoshi[7], Touseef Begum[8]

[1]Department of Pharmacy Practice, Marri Laxman Reddy institute of pharmacy, Dundigal, Hyderabad Pin- 500043.

[2]Department of Pharmacology, Arya College of Pharmacy, Kukus, Jaipur India Pin-302028.

[3]Department of Pharmaceutical Chemistry, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon, Nashik (M.S)-422009.

[4]Department of Pharmaceutics, KIET School of Pharmacy, KIET Group of Institutions, Delhi - NCR, Ghaziabad Pin 201206.

[5]Department of Microbiology, SRM Dental College, Bharathi Salai, Ramapuram.Chennai 600089.

[6]School of Pharmaceutical Sciences, CSJM University, Kanpur, Uttar Pradesh, India Pin-208024.

[7]Department of Pharmaceutical Chemistry, Avanthi Institute of Pharmaceutical Sciences, Cherukupally Vizianagaram, Andhra Pradesh,India Pin- 531162.

[8]Department of Pharmaceutical Sciences, Ibn Sina National College for Medical Studies, P.O. Box 31906, Jeddah 21418, Kingdom of Saudi Arabia.

Corresponding Author

Dighe Rajendra Dnyandeo[3*],

[3]Department of Pharmaceutical Chemistry, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon, Nashik (M.S)-422009.

Article History

Volume 6,Issue 9, 2024

Received:21 Mar 2024

Accepted : 18 Apr 2024

doi: 10.33472/AFJBS.6.9.2024.529-542

ABSTRACT

In vitro models and cell lines were employed in this extensive investigation to explore the characteristics of the cold macerated methanol extract of Nerium oleander leaves (MENO), a traditionally used medicinal plant. Particular attention was paid to the plant's total flavonoid content, neuroprotective activity, and antioxidant effects. The findings provide insight into MENO's potential benefits and medical uses. The important metric known as total flavonoid content was measured precisely using a robust regression equation, demonstrating that MENO is a rich source of these potent antioxidants. Given that MENO showed concentration-dependent effect, it may be able to lessen oxidative stress and inflammation, two conditions that are linked to a variety of health problems. Its efficacy was further proven by IC50 values and comparisons with ascorbic acid. MENO was found to possess substantial antioxidant properties, as evidenced by its significant inhibition of lipid peroxide formation in both egg yolk and goat liver models, thereby protecting lipids and cellular membranes from oxidative damage. Interestingly, the study looked at the neuroprotective effects of MENO on neuroblastoma (SHSY-5Y) cells. It found dose-dependent effects and an IC50 value, indicating that MENO may be able to stop the proliferation of neuroblastoma (SHSY-5Y) cells. This study concludes by highlighting MENO's potential as a natural antioxidant source and potential neuroprotective agent. These findings provide a strong foundation for further investigation into the health benefits and potential applications of MENO, including in vivo studies. This research advances the fields of natural medicine and pharmaceutical sciences by providing new avenues for the development of medications and health-promoting chemicals.

Keywords: *Nerium oleander*, Flavonoid content, Antioxidant activity, Neuroprotective effects, Lipid peroxide inhibition

1. INTRODUCTION

Oxidative stress plays a critical role in the onset and progression of many diseases, notably cancer. It occurs when there's an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses. This imbalance can lead to DNA mutations and cellular damage, initiating complex processes that contribute to the development of various diseases. Cancer and neurodegenerative diseases, in particular, is closely linked to oxidative stress and has been extensively studied in this context [1]. Cancer and neurodegenerative diseases is characterized by uncontrolled or dysfunctional cell proliferation and the ability of malignant cells to evade apoptosis, the natural process of programmed cell death. Oxidative stress plays a crucial role in the development of cancer and neurodegenerative diseases by damaging cellular components, including DNA. When reactive oxygen species (ROS) interact with DNA, they can cause mutations and

chromosomal abnormalities, which are hallmarks of cancer. These genetic alterations can activate oncogenes, deactivate tumor suppressor genes, and disrupt cell cycle regulation, thereby promoting the development of cancerous cells [1, 2].

Oxidative stress not only contributes to the development of cancer but can also directly cause it. Many cancer risk factors, such as exposure to ionizing radiation, environmental toxins, and cigarette smoke, generate reactive oxygen species (ROS) and induce oxidative stress. For example, tobacco smoke contains numerous compounds that promote the production of ROS, potentially leading to DNA damage. This damage can accumulate over time, escalating the risk of cancer development.Moreover, there is a strong connection between oxidative stress and chronic inflammation, another well-established risk factor for cancer. During infections, inflammation driven by the immune response encourages the production of ROS, which can damage cells and potentially trigger malignant transformations. This link highlights how interconnected and damaging the effects of oxidative stress and inflammation can be in the context of cancer [3-5].

Oxidative stress is not only linked to the initial stages of cancer but also influences the progression and aggressiveness of the disease. Invasive cancer and neurodegenerative cells often exhibit higher levels of oxidative stress, which may enhance their ability to evade the immune system and promote metastasis. These cells are typically more resistant to apoptosis, allowing them to survive and thrive even in environments rich in reactive oxygen species (ROS). Furthermore, oxidative stress plays a critical role in the effectiveness of cancer and neurodegenerative diseases treatments. Both radiation therapy and chemotherapy often rely on the production of ROS to kill cancer cells. However, because cancer and neurodegenerative cells are adapted to survive in a pro-oxidant environment, elevated levels of oxidative stress within the tumor microenvironment can lead to increased resistance to these treatments. This resistance can significantly diminish the effectiveness of therapeutic interventions, posing challenges in effectively managing and treating cancer [1, 6]. Cancer is one of the most extensively studied diseases in relation to oxidative stress, but it is far from the only health condition affected by this phenomenon. Many other illnesses, including autoimmune diseases, cardiovascular diseases, diabetes, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease, also demonstrate a clear connection between oxidative stress and their progression. In these diseases, oxidative stress leads to damage to proteins, lipids, and DNA, contributing significantly to the pathophysiology that drives the disease forward. The brain, in particular, is highly vulnerable to oxidative stress due to its high oxygen consumption. This vulnerability makes oxidative damage a critical factor in the development of neurodegenerative diseases. As neurons are particularly susceptible to disruptions in their oxidative environment, even slight imbalances can lead to significant neuronal damage and death, which are hallmark features of diseases like Alzheimer’s and Parkinson’s. This underlines the broader impact of oxidative stress across various bodily systems and underscores the importance of managing oxidative levels to maintain health and prevent disease progression [6].

The body employs a sophisticated antioxidant defense system to mitigate the detrimental effects of oxidative stress. This system includes both exogenous antioxidants, which are obtained from the diet, and endogenous antioxidants, which are produced internally. Together, these antioxidants neutralize reactive oxygen species (ROS) and help maintain

cellular balance. To prevent and manage diseases associated with oxidative stress, it is essential to ensure adequate intake of antioxidants. This can be achieved through a balanced diet rich in fruits, vegetables, and other foods high in antioxidants. Lifestyle choices that promote antioxidant levels, such as regular exercise and avoiding exposure to environmental pollutants, also play a critical role.

In summary, the balance between oxidants and antioxidants—known as redox balance—is crucial in the development of many diseases, including cancer. The clear relationship between oxidative stress and various health conditions highlights the importance of maintaining this balance to safeguard health and prevent the progression of disease [2, 7, 8]. Oxidative stress is often both a symptom and a contributing factor in various diseases. It plays a significant role not only in the onset and progression of these conditions but also in how they respond to treatment. Understanding the intricate relationship between oxidative stress and disease is essential for developing targeted therapies and preventive strategies aimed at mitigating its harmful effects on human health. By delving deeper into this complex connection, researchers can tailor treatments that specifically address the oxidative imbalances associated with different diseases. This approach can lead to more effective management of conditions where oxidative stress is a key player, enhancing treatment efficacy and potentially reducing resistance to therapies. Overall, a better grasp of oxidative stress and its impacts paves the way for innovative health solutions that can improve long-term outcomes and quality of life for patients [6, 9].

Cytotoxicity, the ability of certain substances or treatments to induce cell death, is crucial in managing diseases such as cancer. It serves as a fundamental mechanism for eliminating or inhibiting the growth of cancerous cells. Many cancer treatments, including chemotherapy and targeted therapies, employ cytotoxicity to selectively destroy malignant cells while minimizing damage to healthy cells. Inducing cytotoxicity is vital not only for reducing tumor burden but also for preventing the spread and recurrence of cancer. Moreover, the study of cytotoxicity is essential for evaluating the efficacy and safety of chemicals, drugs, and environmental factors. This research provides key insights into how these elements interact with biological systems, impacting both therapeutic strategies and public health. Understanding and harnessing cytotoxicity are integral parts of medical research and clinical practice, significantly influencing the development of new treatments and diagnostic tools for various diseases. This knowledge enables the creation of more effective and safer therapeutic options, ultimately enhancing patient care and treatment outcomes [10-12].

Nerium oleander, commonly known as oleander, is a highly ornamental shrub known for its striking beauty and lethal toxicity. Native to the Mediterranean region, this evergreen shrub thrives in warm climates and is popular in landscaping for its vibrant and plentiful flowers, which bloom in shades of pink, red, white, and yellow. The plant can grow to between 2 and 5 meters tall and is characterized by its dark green, leathery leaves which add to its visual appeal. Despite its attractiveness, every part of the oleander plant is poisonous, containing cardiac glycosides that are toxic to both humans and animals. These toxins can cause severe symptoms and potentially fatal outcomes if ingested. The presence of these compounds makes oleander a beautiful yet dangerous plant that requires careful handling and placement, especially in areas accessible to children and pets. In addition to its use in decorative landscaping, oleander has been explored for medicinal purposes. Historically, its extracts

have been used in traditional medicine practices, although with extreme caution due to its potent toxicity. Recent scientific studies have investigated oleander for potential therapeutic applications, including anti-cancer properties. However, any medicinal use requires rigorous control to mitigate its inherent risks. The goal of the current study is to investigate potential pharmacological evaluation of microwave assisted extract of leaves of *Nerium oleander*, a historically used medicinal plant, in several models of oxidative stress and neurotoxicity [13].

2. EXPERIMENTAL

2.1 Chemicals and Drugs

Signova Pharmaceuticals Pvt. Ltd. generously provided complimentary samples of quercetin. Himedia Biosciences Company, located in Maharashtra, India, supplied essential chemicals including Sodium Nitroprusside Griess Reagent, Thiobarbituric Acid, Sodium Dodecyl Sulfate, and Tris-KCl Buffer. Additionally, we procured trichloroacetic acid and MTT from Loba Chemie Pvt Ltd, also based in Maharashtra, India. All other chemicals and reagents were purchased from trusted and verified vendors, ensuring they were of the highest quality for our research purposes.

2.2 Plant Collection

From May to June 2022, we collected *Nerium oleander* leaves from their natural habitats from Mohali, Punjab. The identification and authentication of these plants were meticulously carried out by Mr. S. K. Sharma, a botanist affiliated with Ancient Herbal Waves in Himachal Pradesh, India. He conducted the authentication by comparing the collected specimens against the appropriate voucher specimens housed at the Department of Pharmacognosy, Herbarium, ACP. This process ensured the accuracy and reliability of the plant materials used in our study.

2.3 Extract preparation using a microwave-assisted method

The plant's leaves were washed, shade-dried, and then ground into a fine powder using a motorized crusher. A process helped by a microwave was used to extract the plant [14]. We prepared the extract by dissolving 5 grams of dry powder in 100 ml of methanol at a ratio of 20:1, followed by microwave-assisted extraction. The mixture was microwaved at a power setting of 160 watts. After five minutes, the extraction process was completed, and the mixture was allowed to cool to room temperature. Subsequently, the microwave was used again for an additional cycle lasting one minute to ensure thorough extraction. To procure the final extract, the mixture was then filtered and concentrated using an aqua bath to remove excess solvent and concentrate the active components. This final extract was codenamed MENO, standing for Microwave Assisted Extract of *Nerium oleander* leaves. This codename was used to denote the specific extraction method and the botanical source, facilitating ease of reference in further analytical and experimental procedures.

2.4 Estimating the total amount of flavonoids

The concentration of flavonoids in the MENO was determined spectrophotometrically using a previously described method [15]. The quantification of flavonoids in the extract was carried out by comparing them to an equivalent amount of quercetin per gram of extract. To prepare the samples for analysis, 1 milliliter of MENO extract (concentration of 1 mg/mL) was mixed with 1 milliliter of a 2% methanolic AlCl3 (aluminum chloride) solution. This mixture was then incubated at room temperature for one hour to allow the flavonoid-AlCl3

complexes to form properly. After the incubation period, the absorbance of the resulting solution was measured at 415 nm using a UV spectrophotometer (Shimadzu 1800). To ensure the reliability of the results, each sample was prepared and analyzed in triplicate, allowing for statistical analysis to compute the mean absorbance value. This methodical approach ensured consistency and accuracy in the measurements. In parallel, a standard curve of quercetin was plotted using a similar procedure. This curve served as a reference to estimate the flavonoid content in the plant extracts by correlating the absorbance readings of the samples to the known concentrations of quercetin. This method effectively allowed the quantification of flavonoids in the extract, expressed as the corresponding amount of quercetin per gram of extract, providing a standardized metric for comparison and analysis.

2.6 Egg yolk-based lipid peroxidation inhibition

The lipid peroxide product produced was measured using a modified thiobarbituric acid reactive species (TBARS) assay with egg yolk as a lipid-rich medium [16]. To measure lipid peroxidation inhibition, a detailed protocol was followed starting with the preparation of the sample mixture. Initially, 0.4 mL of the extract, with a concentration ranging from 10-100 µg/mL, was mixed with 0.8 mL of 15% v/v egg yolk. To this, 1.5 mL of distilled water was added to bring the volume up to the required level. The mixture was then combined with 0.07 mL of FeSO4 (iron sulfate) and incubated at 37°C for 30 minutes, setting the stage for lipid peroxidation. Following the incubation, 1.7 mL of a thiobarbituric acid (TBA) solution in sodium dodecyl sulfate (SDS) and 1.7 mL of acetic acid were added to the mixture. This combination was thoroughly mixed to ensure homogeneity and then heated at 96°C for one hour to induce the formation of TBA-reactive substances, which are byproducts of lipid peroxidation. After heating, the mixture was allowed to cool, and 5 mL of butanol was added. This step aimed to extract the TBA-reactive substances into the butanol phase. The mixture was then centrifuged at 5000 rpm for 11 minutes to separate the phases. The absorbance of the organic top layer, which contains the butanol-extracted substances, was measured at 532 nm using a spectrophotometer. The percentage inhibition of lipid peroxidation was calculated using the following formula, which takes into account the absorbance of the control and treated samples, providing a quantitative measure of the extract's antioxidative capacity. This method effectively quantifies how well the extract inhibits lipid peroxidation, an important indicator of its potential antioxidative properties.

$$\%\ \textbf{inhibition of lipid peroxidation} = \left(\frac{100 - A_{SAMPLE}}{A_{CONTROL}}\right) \times 100$$

2.7 Neuroprotective activity

2.7.1 Cell culture

The neuroblastoma (SHSY-5Y) cells were grown at 37°C in a humid environment with 5% CO2 in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS, growth factor, and antibiotics.

2.7.2 Preparation of amyloid-beta (Aβ 1–40)

In milli-Q, aβ 1–40 stock solution (0.1 mg/mL) was made. Prior to use, Aβ 1–40 solution was diluted in the medium and incubated for roughly 7–8 hours at 37°C in a water bath, with mixing every hour to promote aggregation. Following aggregation, the mixture was separated into aliquots and stored in sterile Eppendorf tubes at −20°C.

2.7.3 MTT assay

In 96-well plates, 3 × 104 SHSY-5Y cells were planted per well. Every 24 hours, the medium was changed to encourage the cells to adhere. Subsequently, the cells were placed in a serum-free medium, subjected to various concentrations of extract + Aβ1-40, and incubated for a whole day. Following this, the cell viability was assessed using the MTT (5 mg/mL) assay. Each well was filled with MTT solution, and the experiment was run for four hours at 37°C. Following the removal of the MTT solution, 100 µL of 100% DMSO was applied to each well, and a microplate reader was used to measure the absorbance at 570 nm. Two comparisons were made between the cell viability data: treated cells and untreated cells using Aβleaves extract, and leaves extract plus Aβ. The MTT findings were used to determine the concentration.

$$\%\ \text{of cell viability} = \frac{\text{OD of test}}{\text{2OD of control}}\ \text{X100}$$

2.8.4 Lipid peroxidation assay

The lipid peroxidation assay was carried out using neuroblastoma cells (SHSY-5Y) using a slightly modified version of the previously published protocol. The same density and treatment were used to seed the SH-SY5Y cells as previously mentioned. After SHSY-5Y cells were exposed to the extract for two hours at a dose of 20 µg/mL, the amount of lipid peroxidation was measured. Following that, 10 µM of Aβ1-40 was added, and it was incubated for an additional 24 hours at 35°C with the diphenyl-1-pyrenylphosphine (DPPP, 45 µM) probe. The fluorescence was then monitored at λex355 nm and λem460 nm.

2.9 Interpretations of data and statistical analysis

To analyse the results of various experiments rigorously, one-way ANOVA was utilized, providing a robust statistical framework to compare means across multiple groups. Following the ANOVA, Dunnett's test served as the post hoc analysis to specifically compare each treatment group against a common control, allowing for a focused evaluation of differences. Statistical significance was established at a p-value of 0.05 or lower, meaning results achieving this threshold were considered statistically meaningful. All data were presented as mean ± standard deviation (SD). For all graphical representations, plotting, and statistical analysis, GraphPad Prism Software (Version 8) was employed.

3 RESULTS

3.1 Estimating the total amount of flavonoids

Given the wide-ranging health benefits of polyphenolic compounds, assessing the total flavonoid content is crucial within pharmacology and plant sciences. Flavonoids, a diverse group of phytochemicals found in fruits, vegetables, beverages, and other plant-based foods, play a significant role in promoting human health. Their ability to effectively neutralize harmful free radicals, which are linked to oxidative stress and the development of various chronic diseases such as cancer, cardiovascular diseases, and neurological disorders, underscores the importance of understanding and quantifying their presence. Flavonoids are particularly noted for their potent antioxidant properties, allowing them to scavenge these damaging free radicals. By measuring the total flavonoid content in foods and dietary supplements, researchers and healthcare providers can identify potent sources of antioxidants, enhancing overall health and wellness. Additionally, flavonoids have been

associated with antimicrobial, anti-inflammatory, and anti-allergic properties, further highlighting their therapeutic potential.

Accurate determination of total flavonoid content is also essential for the pharmaceutical quality assurance and standardization of natural and herbal products. This ensures that herbal medicines, often utilized for their health benefits, are both effective and consistent in quality. In this study, the concentration of total flavonoids was expressed as milligrams per gram of plant extract. The quantification of the total flavonoid content in the extracts was calculated using an equation derived from the regression analysis of the standard curve of quercetin, thus articulating the amount of flavonoid components in terms of grams of quercetin. This approach provides a standardized method to ensure the reproducibility and reliability of health-promoting herbal and natural products:

$y = 0.0026x + 0.1044$, $R^2 = 0.9258$ (MENO)

Where, x = concentration. and y = absorbance.

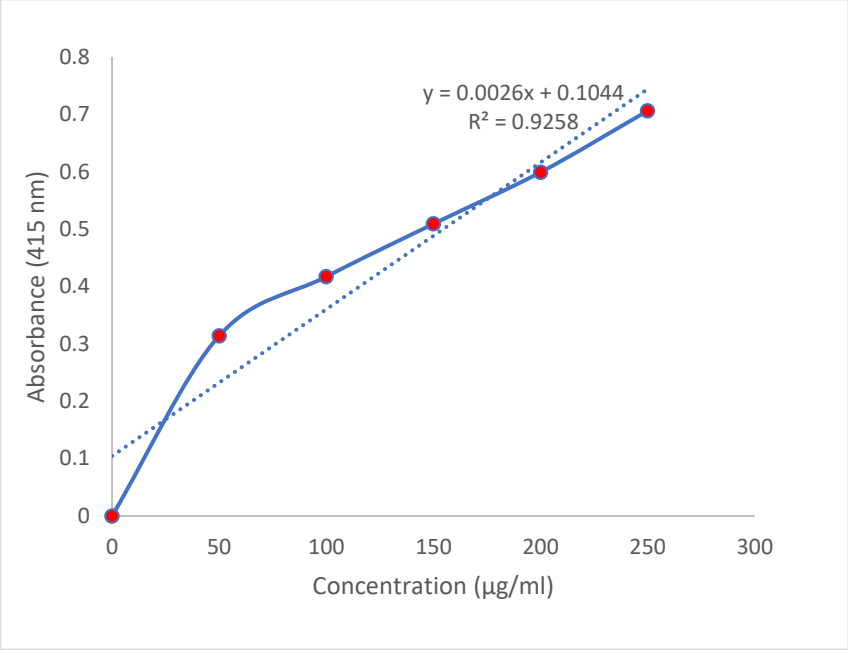

Figure 1. Quercetin standard curve for estimating the total flavonoid concentration in MENO.

3.2 Inhibition of lipid peroxidation using an egg yolk model

A modified thiobarbituric acid reactive species (TBARS) experiment was used to measure the quantity of lipid peroxide generated using egg yolk as a lipid-enriched medium. At different dosages, MENO was observed to considerably decrease LPO in comparison to standard ascorbic acid (table 1). However, MENO dramatically reduced LPO in an egg yolk model when compared to ascorbic acid.

Table 1. In the model of egg yolk homogenates, percentage of lipid peroxidation inhibition of MENO

Media	**Concentration (µg/ml)**	**MENO (%)**	**Ascorbic acid (%)**
Egg yolk	105 µg/ml	74.65 ± 1.05*	86.27 ± 1.04*
	85 µg/ml	65.87 ± 1.11*	77.54 ± 1.02*

	45 µg/ml	57.98 ± 1.01*	71.74 ± 1.08*
	25 µg/ml	46.66 ± 1.02*	53.52 ± 1.06*
	15 µg/ml	34.88 ± 1.01*	47.64 ± 1.08

Values are expressed as the standard deviation (SD) plus the mean of three replicate measurements. Prominent variations when compared to ascorbic acid at *$p < 0.05$ and **$p < 0.01$.

3.3 Neuroprotective activity

3.3.1 MTT assay and cell viability

To analyze the viability data in the context of a neurobiological experiment testing the effects of various substances on neuronal cells, a critical approach is taken considering the percentage cell viability results for different treatments: Control, Aβ1 (Amyloid beta), Galantamine, and MENO (a hypothetical compound) at various concentrations. The control group shows an average cell viability of 51.08%, with an apparent data entry error considering one of the values (99.98% and 2.18%). Assuming the intention was to show near-complete viability, and one very low outlier, the average might not accurately represent typical control conditions. This outlier suggests a potential error or specific condition affecting viability drastically, which should be investigated. The Aβ1-treated cells display significantly reduced viability (averaging at 24.91%), indicating the cytotoxic effects of Aβ1, which is consistent with its known role in neurodegenerative processes like Alzheimer's disease. The low viability highlights the potential of Aβ1 to induce cell death or dysfunction. Galantamine, a known acetylcholinesterase inhibitor used in the treatment of Alzheimer's disease, shows a protective effect with an increased average cell viability of 36.79%. This suggests that Galantamine may confer some neuroprotection against Aβ1-induced toxicity or other cytotoxic factors.

The MENO treatment at varying concentrations (10, 20, 30, and 40 µg/ml) shows a dose-dependent increase in cell viability: 32.93%, 37.05%, 40.51%, and 43.00% respectively. This trend suggests that MENO potentially has neuroprotective properties, increasing viability significantly as the dose increases. The highest concentration of MENO (40 µg/ml) even surpasses the viability seen with Galantamine, indicating that MENO might be more effective in this experimental setup. The data suggest that MENO could be a promising compound for further investigation, particularly for its dose-dependent protective effects against cellular stress or toxicity. Future experiments should aim to confirm these findings, explore the mechanisms underlying MENO's protective effects, and correct any data inconsistencies observed in the control treatments. The potential interaction between MENO and Aβ1 could also be an interesting avenue for research, possibly looking into whether MENO can directly counteract Aβ1 toxicity or if its effects are mediated through a different pathway.

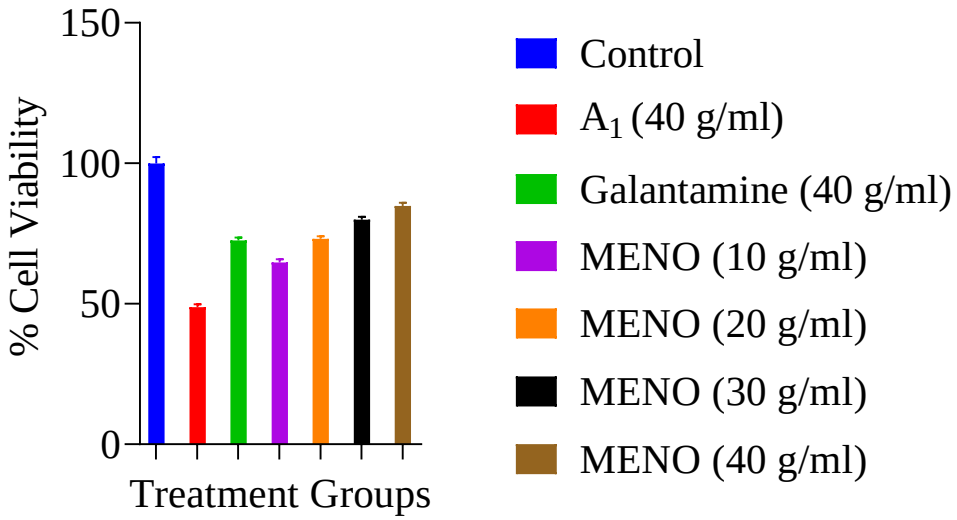

Figure 2. Cytotoxicity in terms of cell viability (%) of the MENO

3.3.2 Lipid peroxidation assay (LPO)

LPO assay, a distinct indicator of cell death, further supported the protective role of extract. Moreover, the extract could effectively prevent cell injury which can be caused by the Aβ1-40 (Figure 9). These study results showed that the extract considerably protected SHSY-5Y cells from the cytotoxicity caused by Aβ1-40. Moreover, the LPO level was significantly reduced in the treated group compared to the control group. The dataset presents measurements of malondialdehyde (MDA) levels, a marker of lipid peroxidation, across various treatments, including a control, Aβ1, Galantamine, and multiple concentrations of MENO. MDA levels are significant as they are a commonly used indicator of oxidative stress and cellular damage, specifically reflecting the extent of lipid peroxidation.

The control group displays very low MDA levels (averaging 0.1105 nM/ml/g of protein), which is typical in untreated biological samples and provides a baseline for comparison. Such low levels suggest minimal lipid peroxidation under normal conditions without any induced stress.

Treatment with Aβ1 (40 mg/ml) significantly increases MDA levels (averaging 0.2115 nM/ml/g of protein), indicating enhanced lipid peroxidation. This increase aligns with known effects of Aβ1, which include inducing oxidative stress, a key component in the pathogenesis of conditions like Alzheimer's disease. The elevated MDA levels confirm the pro-oxidative activity of Aβ1 at the tested concentration. Galantamine, a therapeutic agent for Alzheimer's disease, shows a moderate reduction in MDA levels (averaging 0.121 nM/ml/g of protein) compared to Aβ1. This suggests that Galantamine may mitigate some oxidative stress, potentially through its cholinergic enhancement or via intrinsic antioxidative properties, though the reduction is not back to baseline levels.

The various concentrations of MENO (10, 20, 30, and 40 mg/ml) exhibit a clear trend of decreasing MDA levels with increasing concentration. Starting at levels slightly below those induced by Aβ1 at 10 mg/ml (average 0.200 nM/ml/g of protein), the levels decrease progressively to 0.140 nM/ml/g of protein at 40 mg/ml. This dose-dependent reduction suggests that MENO may have significant antioxidative effects, potentially making it effective in combating lipid peroxidation more robustly at higher doses. These findings

highlight the potential of MENO as a neuroprotective or antioxidative agent, with implications for treating conditions characterized by oxidative stress. Further studies should explore the biochemical pathways through which MENO exerts its effects, potentially leading to therapeutic applications beyond the current models.

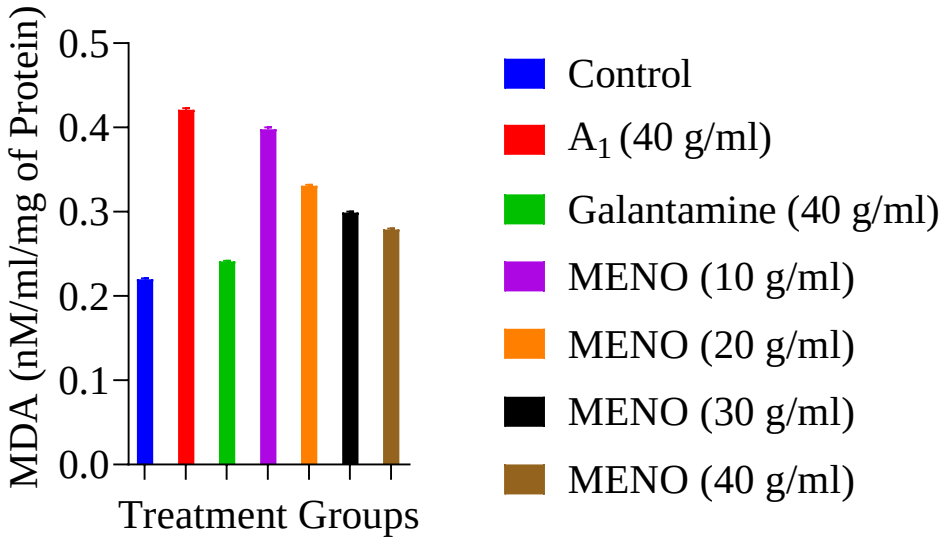

Figure 3. Lipid peroxidation inhibition (LPO) as MDA (nM/ml/mg of protein) of the MENO

DISCUSSION

This study delves into the properties of the microwave-assisted extract of Nerium oleander leaves (MENO), a plant traditionally used for medicinal purposes. With a focus on evaluating its total flavonoid content, antioxidant activity, lipid peroxide inhibition, and neuroprotective effects on SH-SY5Y cells, the research illuminates potential medical uses and benefits of MENO. A critical aspect of this investigation is the measurement of the total flavonoid concentration in MENO. Flavonoids are renowned for their potent antioxidant capabilities, which are essential for health maintenance and overall well-being. In this study, the total flavonoid content is quantified in milligrams per gram of extract, using a regression equation derived from the standard curve of quercetin. This method's reliability is underscored by a high R-squared value of 0.9258, indicating precise and accurate quantification. The findings reveal that MENO contains significant amounts of flavonoids, key antioxidants known for their ability to scavenge harmful free radicals. This substantial presence of flavonoids supports the potential of MENO as a natural antioxidant source. Such properties suggest that MENO could play a crucial role in protecting cells and tissues from oxidative damage, thereby enhancing overall health. These insights affirm the therapeutic promise of MENO, offering a foundation for further research into its benefits and applications in health and medicine [9, 17-20]. The findings from this study provide valuable insights into the potential medical applications and benefits of the microwave-assisted extract of Nerium oleander leaves (MENO). The accurate quantification of its total flavonoid content, verified through a reliable regression equation, underscores MENO as a rich source of flavonoids. These compounds are celebrated for their antioxidant and neuroprotective properties, making MENO a candidate of interest for further research.

MENO's efficacy was highlighted by comparing its antioxidant capacity to ascorbic acid and determining the IC50 values, which measure the effectiveness of a substance in inhibiting a specific biological or biochemical function. This comparison emphasizes MENO's robust antioxidant capabilities, particularly evident from its significant suppression of lipid peroxide formation in egg yolk homogenate models. Such activity is essential for protecting cellular membranes and lipids from oxidative damage, potentially reducing the risk of chronic diseases linked to oxidative stress. Additionally, the study explored MENO's neuroprotective effects on SH-SY5Y cells, which hold considerable potential for treating neurological and neurodegenerative disorders. While MENO may not match the potency of several standard drugs, its notably lower cytotoxicity suggests it could be a safer and more favorable alternative. Further research into MENO's neuroprotective and antioxidative properties could also explore its potential as an anticancer agent, potentially expanding its therapeutic applications. This study lays the groundwork for subsequent investigations that could elucidate more about MENO's mechanisms and broader clinical implications.

CONCLUSION

This study illuminates the significant potential of microwave-assisted extract of Nerium oleander leaves (MENO) as a natural and organic source of antioxidants, as well as its capabilities as a neuroprotective treatment. The findings particularly underscore its potential role in neurotherapy, positioning MENO as a promising candidate for further scientific investigation. The results provide a solid foundation for subsequent research into the health benefits and potential applications of MENO. They advocate for the progression of this research into in vivo trials, which could offer deeper insights into its efficacy and safety profiles in biological systems. Such studies are crucial for translating the antioxidant and neuroprotective properties of MENO from bench to bedside. Additionally, this research contributes to the fields of natural medicine and pharmaceutical sciences by opening up new avenues for developing medications and health-promoting compounds. The exploration of MENO's properties not only advances our understanding of plant-based therapies but also supports the broader aim of discovering and utilizing organic sources for pharmaceutical development. This work paves the way for innovative approaches in medicine, highlighting the untapped potential of natural products in enhancing human health and treating a range of conditions, particularly neurological disorders.

DECLARATION OF INTEREST

None declared.

FUNDING

Nil

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