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EVALUATION OF THE NEUROPROTECTIVE POTENTIAL HYDROALCOHOLIC FLOWER EXTRACT OF NERIUM OLEANDER

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ABSTRACT

INTRODUCTION:

Neurodegenerative disorders such as Alzheimer's, Parkinson's diseases etc, pose significant challenges to human health and well being. The search for effective neuroprotective agents to combat these conditions have intensified in recent years. One such potential candidate is the hydroalcoholic flower extract of Nerium oleander, a shrub known for its medicinal properties. It contains a rich array of bio active compounds, including flavonoids and phenolic compounds which have been reported to possess antioxidant, anti inflammatory and neuroprotective properties

AIM:

This study aims to assess the neuroprotective effects of the hydroalcoholic flower extract of Nerium oleander using in vitro experiment model

OBJECTIVES:

The study aims to enhance our understanding of the neuroprotective potential hydroalcoholic flower extract of Nerium oleander and its application in the prevention and treatment of neurodegenerative diseases

MATERIALS AND METHODS:

The flowers of Nerium oleander collected were shade dried and then coarsely powdered. 100 grams of the plant extract was packed in Soxhlet extractor with 200 ml of 70% hydro alcohol. Extraction was carried out using a hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a concentrated material which was used for further assessment)

1. *Xanthine oxidase inhibitory activity*
2. *In vitro acetylcholinesterase inhibitor assay (Ellaman et al., 1961)*
3. *Amyloid peptidase inhibitory action*

RESULTS:

The present study results are in concordance with the earlier published results demonstrating the inhibitory property of Nerium oleander flower extract against xanthine oxidase, acetylcholinesterase and amyloid beta peptidase formation. From this we can suggest that the plant extract is neuroprotective

CONCLUSION:

In conclusion, the evaluation of the neuroprotective potential of the hydroalcoholic flower extract of Nerium oleander holds great promise for the development of novel therapeutic strategies for neurodegenerative disorders.However, further research is warranted to fully understand the underlying mechanisms of action and optimize the therapeutic potential of the hydroalcoholic flower extract.

Keywords: Neuroprotective potential, Hydroalcoholic flower extract, Nerium oleander, Neurodegenerative disorders, Neurological injuries, Neuronal preservation.

INTRODUCTION

Neurodegenerative diseases and neurological wounds present huge difficulties to human wellbeing and prosperity, as they frequently lead to the dynamic loss of neurons and ensuing mental and motor dysfunctions(1). As a result, there is an urgent need to investigate potential therapeutic approaches that have the potential to preserve and protect neurons in the central nervous system (CNS). Due to their potential neuroprotective properties, natural compounds derived from plants have received a lot of attention in recent years. Neuroinflammation has been shown to play a significant role in both acute and chronic neurodegenerative conditions, including stroke, traumatic brain injury (TBI), Parkinson's disease, Alzheimer's disease, multiple sclerosis, and multiple sclerosis (2). They are all connected to the activation of microglia and are accompanied by high levels of proinflammatory mediator expression (3). The progression of Alzheimer's disease (AD), an irreversible neurological condition characterized by cognitive decline, has not been slowed by any of the known treatments (4) . The central nervous system (CNS) uses neuroinflammation as a defense mechanism to ward off infection and injury. Microglia, which are the resident macrophages of the central nervous system (CNS), are the main effector cells in mediating neuroinflammation, which is characterized by the activation of a number of inflammatory and neurodegenerative illnesses.(5).

The flowering shrub Nerium oleander, more commonly referred to as oleander, is a member of the Apocynaceae family. It is found in a lot of different parts of the world and is known for its colorful flowers. Over the entire course of time, Nerium oleander has been utilized in customary medication for the treatment of different diseases, including cardiovascular circumstances, skin problems, and irritation (6). The Nerium oleander plant is drought-tolerant . The dark-green coriaceus blades on the 5 to 20 cm-long, acuminate or acute, shortly petiolate, narrow leaves are Five petals and varying hues of lilac, salmon, carmine, deep to pale pink, copper, apricot, orange, white, and yellow characterize the terminal cluster of approximately 5 cm in diameter. The fruit is made up of a short, 7.5 to 17.5 cm-long follicle that opens to release fluffy seeds. This plant can be proliferated by seed and shows extraordinary fluctuation in seedling populaces.

Nerium oleander is broadly developed as a decorative plant in tropical, subtropical and calm districts because of its abundant blossoming which are enduring alongside their moderate strength (7) . It is used for screening, hedging, and planting along beaches and highways. By leaving only a few stems behind, it is able to grow into attractive small trees. It can be grown indoors or on a patio in the northern regions. In addition, the plant had antibacterial properties, mitigating, antinociceptive and antitumor exercise. This plant's potential therapeutic effects in the neuroscience field, particularly its neuroprotective properties, have also been studied.

The blossoms of Nerium oleander contain a different cluster of bioactive mixtures, including alkaloids, flavonoids, cardiovascular glycosides, terpenoids, and phenolic compounds. These substances have shown a variety of pharmacological activities, including antioxidant, anti-inflammatory, and anti-cancer effects that are known to be important for neuroprotection. (8)Consequently, research into the hydroalcoholic flower extract of Nerium oleander's neuroprotective potential is becoming increasingly popular. The goal of neuroprotection is to slow the progression of neurodegenerative disorders or neurological injuries by preserving and protecting neurons from damage or degeneration. The discovery of natural compounds with the potential to protect neurons is crucial for the development of novel neuroscience-based therapeutic approaches.

MATERIALS AND METHODS:

This study was conducted in Saveetha Dental College, Department of Forensic Odontology. The time duration of the this study was 3 months

Plant material

Fresh flowers of *Nerium oleander* were collected from Chennai, Tamil Nadu. The *N.oleander* flowers were then shade dried at ambient temperature. Thereafter the dried flowers were pulverized into a coarse powder and ready for extraction.

Preparation of plant extract

The flowers of *Nerium oleander* collected were shade dried and then coarsely powdered. About hundred grams of dried *N.oleander* flowers were weighed and packed in a Soxhlet extractor with 200 ml of 70% hydro alcohol (70% ethanol and 30% water). Extraction was carried out using a hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a concentrated material. The extracts were concentrated and used for further experiments.

Chemicals and reagents

Xanthine, acetylthiocholine iodide, acetylcholine enzyme (0.3U/ml) were procured from Sigma-aldrich, USA. Quercetin was purchased from TCI chemicals, India. Donepezil hydrochloride was purchased as a tablet from a local pharmacy. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

Xanthine oxidase inhibitory activity

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions, based on the procedure reported by Bustanji et al. 2011. The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50µL of different concentrations *N.oleander* (10-320µg/ml), different concentrations of Quercetin (10-320µM), 35µL of 0.1mM phosphate buffer (pH=7.5) and 30µL of enzyme solution (0.01units/ml of XO in 0.1mM phosphate buffer, pH=7.5), was prepared immediately before use. After 30 mins of incubation at 25^{o}C, the reaction was initiated by the addition of 60µL of substrate solution (150mM of Xanthine in 0.1mM Phosphate buffer). The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. A blank was prepared in the same manner. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid/minute at 25 °C. XO inhibitory activity is expressed as the percentage inhibition of XO in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of *N.oleander* and Quercetin. IC_{50} values were calculated from the mean values of data from three determinations. Quercetin was used as a reference standard.

***In vitro* acetylcholinesterase (AChE) inhibition assay (Ellman et al., 1961)**

The hydroalcoholic extract of *N.oleander* and standard Donepezil hydrochloride was examined for its AChE inhibitory activities at different concentrations of 10-320µM and 10-320µg/ml respectively. 200µl of the different concentrations *N.oleander* (10-320µg/ml) and standard Donepezil hydrochloride (10-320µg/ml) were prepared using 0.05M tris base. Briefly, in this method, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of *N.oleander* extract and Donezepil at different concentrations were mixed and incubated for 15 min at 30ºC. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200µl of AChE (0.3U/ml) solution were added to the initial mixture, to start the reaction and then the absorbance was determined.

Control contained all components except the tested extract. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

IA (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100

Assessment of A*β* (1–42) Concentration

Preparation of Aβ solution

The Aβ solution was prepared according to the method of Miyazaki et al., 2019. Briefly, synthetic β-Amyloid Peptide 1-42 (Aβ1-42) (PP69, Sigma Merck, USA) was dissolved in 0.1% ammonia solution at a final concentration of 250 µM and sonicated in ice-cold water for a total of 5 min (1 min × 5 times) to avoid pre-aggregation. For preparation of the Aβ solution, aliquots of Aβ were diluted to 25µM in 50mM phosphate buffer (pH 7.5) and 100mM NaCl.

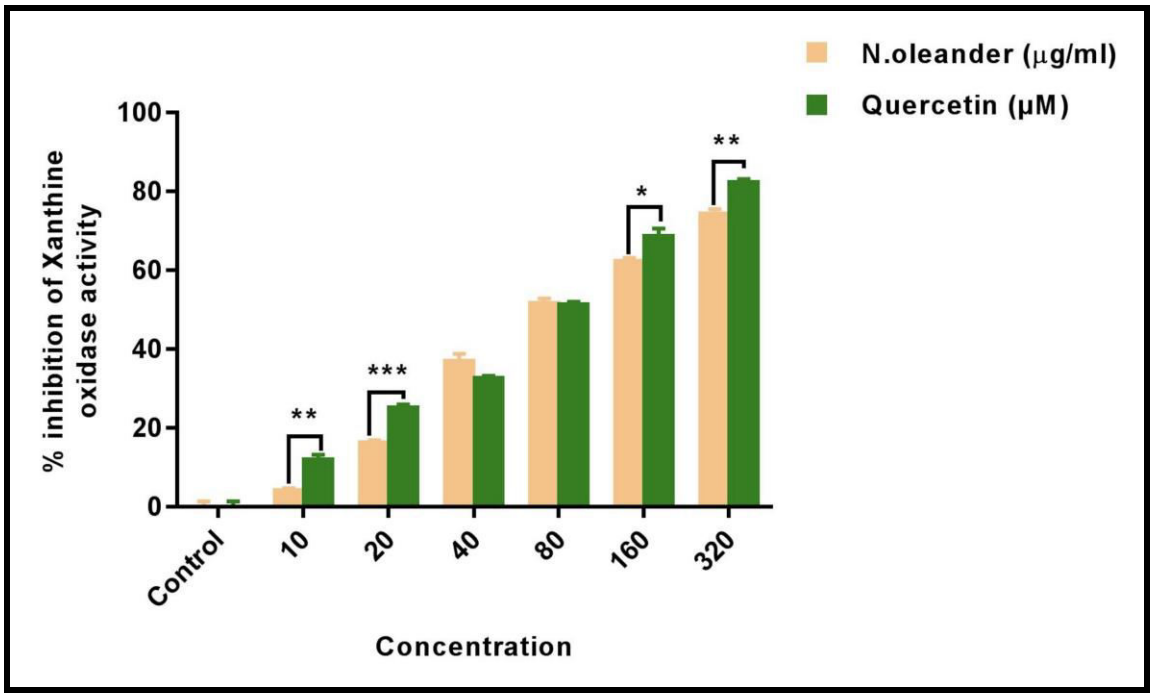
Thioflavin T fluorescence assay

The thioflavin T (ThT) fluorescence assay was performed as Miyazaki et al., 2019. Aβ solution (8µL) was mixed with the different concentrations of *N.oleander* (10-320µg/ml) and Donepezil (10-320µg/ml) and the mixture was then added to 1.6mL of ThT solution containing 5µM ThT and 50mM NaOH-glycine-buffer (pH 8.5). The samples were incubated at 37°C and the fibrillogenesis rate was monitored by using ThT fluorescence assays. The samples ThT fluorescence levels were evaluated by using Biotek Synergy H4 hybrid multi mode reader (USA). The respective excitation and emission wavelengths were 446 nm and 490 nm.

Statistical analysis

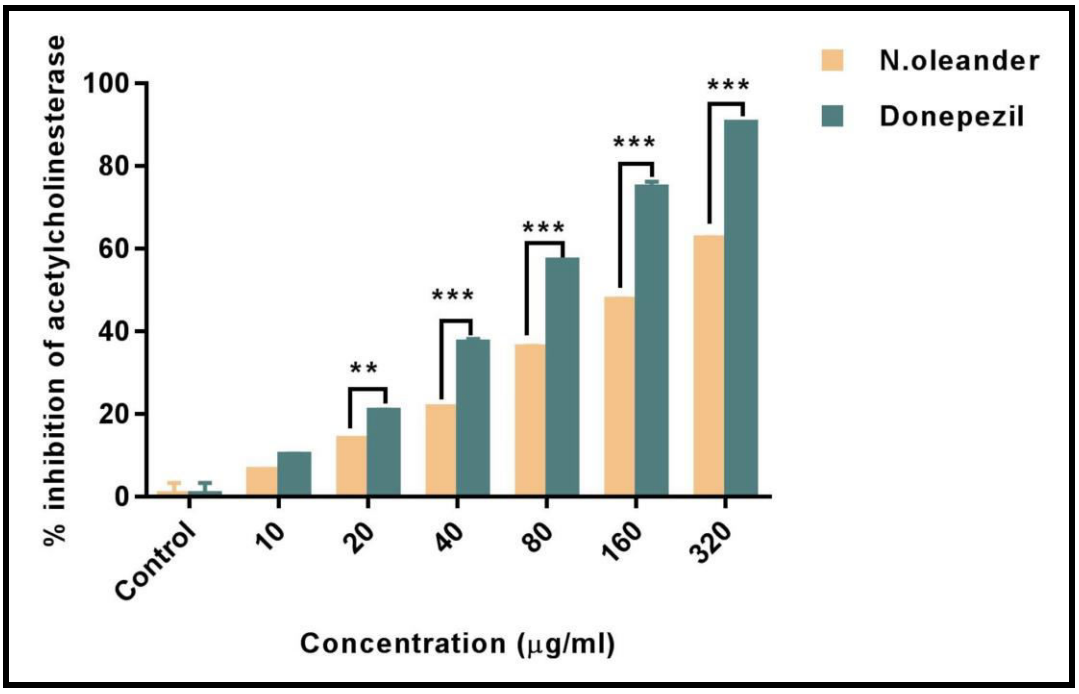
Data were analyzed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC_{50} values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standard groups using the Holm-Sidak Test.

RESULTS



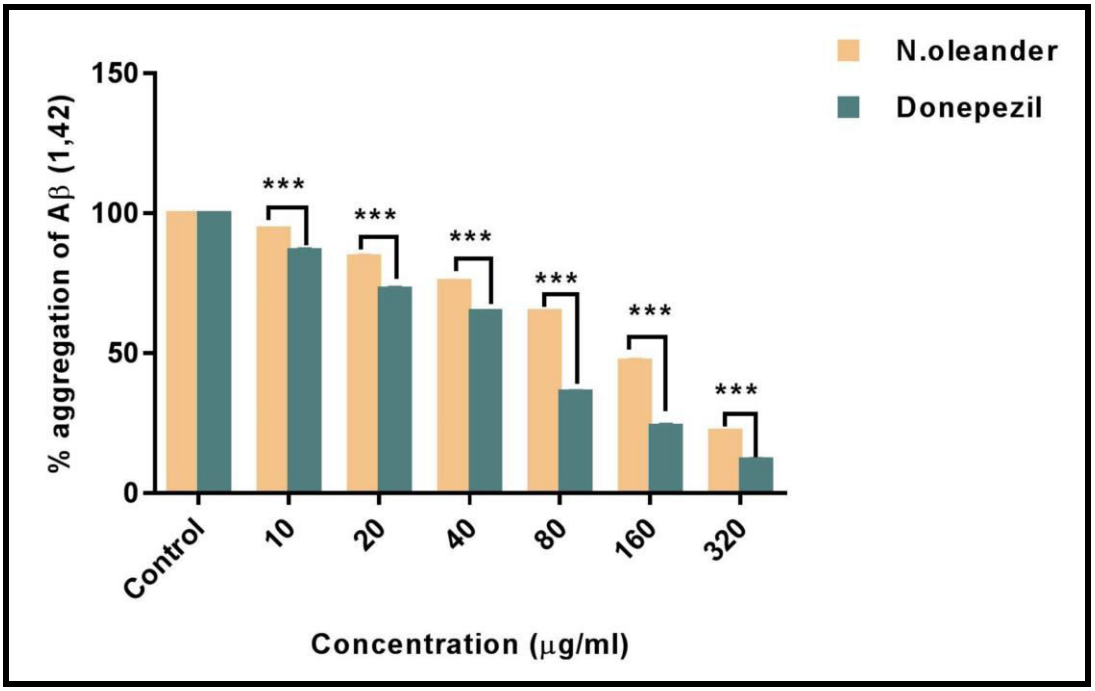
GRAPH 1: Represents the percentage inhibition of Xanthine oxidase activity

X - axis here represents the concentration of N. oleander and quercetin. The y - axis represents the % inhibition of xanthine oxidase activity



GRAPH 2: Represents the percentage inhibition of acetylcholinesterase

X - axis in this graph represents the concentration of N. oleander and donepezil and the Y- axis represents % inhibition of acetylcholinesterase



GRAPH 3: Represents the percentage aggregation of amyloid beta plaque formation

X - axis indicates the concentration of N. oleander and donepezil and the Y - axis represents the %aggregation of amyloid beta plaque formation

DISCUSSION

The x - axis of graph 1 represents the concentration of Nerium oleander and Quercetin and the y- axis represents the percentage inhibition of xanthine oxidase activity. It is evident that as the concentration increases the inhibition percentage also increases. Xanthine oxidase inhibitors are frequently employed in the management of hyperuricemic illnesses such as gout, nephropathy, and renal stone disease. The possibility that these medications might be useful in preventing vascular diseases, particularly those that affect the cerebrovasculature, has recently attracted attention. This interest has been sparked by new research that suggests serum uric acid may play a role in the development of cardiovascular disease. Additionally, because xanthine oxidase inhibition may have advantages over uric acid reduction because it causes oxidative stress in the vasculature, this interest has been sparked.(9)

In graph 2, the percentage of inhibition by the Nerium oleander and Donepezil against acetylcholinesterase was checked. It was found that the percentage inhibition of donepezil is greater compared to the N. oleander as the concentration gradually increases.. The medicinal acetylcholinesterase (AChE) inhibitors donepezil, galantamine, and tacrine that are now used to treat Alzheimer's disease also shielded neuronal cells from glutamate neurotoxicity. Nicotine and AChE inhibitors' protective effects were countered by nAChR antagonists.(10)

In graph 3, the graph represents the percentage aggregation of amyloid beta plaque formation by donepezil and Nerium oleander. As concentration increases, the aggression of donepezil is less compared to N. oleander. Neurofibrillary tangles and aberrant neuritic plaque buildup are hallmarks of Alzheimer's disease. Plaques are spherical microscopic lesions that feature an enlarging axonal end and an extracellular amyloid beta-peptide core.(11)

Although Nerium oleander is not as effective as the commercially available drugs, it can be used as a herbal alternative to prevent neurodegenerative disorders

The present study results are in concordance with the earlier published results demonstrating the inhibitory property of Nerium oleander flower extract against xanthine oxidase, acetylcholinesterase and amyloid beta peptidase formation. From this we can suggest that the plant extract is neuroprotective

In a previous study conducted by Kamran et al 2015, Plant extracts were used to synthesize inexpensive, environmentally acceptable, and easily scalable metallic nanoparticles. A regulated size and form of nanoparticles may be produced using the plant extract. In this paper, they describe the fabrication of gold nanoparticles utilizing aqueous N. oleander leaf extracts, which serve as both reducing and stabilizing agents. The biogenic gold nanoparticles are tiny and almost spherical in form, with no signs of aggregation. The created gold nanoparticles demonstrated effective antioxidant action. (12)

According to a specific study done in 2012 by Kumar et al., the extract had strong reducing power, lipid peroxide, DPPH, ABTS, superoxide anion, hydroxyl radical, and metal chelation activities. The MENO-F (methanolic extract of flowers of Nerium oleander) was observed to significantly restore the significantly raised serum enzymatic levels of AST, ALT, ALP, and total bilirubin toward normality in a dose-dependent manner, with maximum hepatoprotection at the 400 mg/kg dose level. The biochemical proof of hepatoprotection was supported by the histological findings. The hepatoprotective results are further strengthened by increased levels of SOD and lower levels of MDA. According to the findings of the investigation, MENO-F possesses strong antioxidant and hepatoprotective properties against CCl4-induced liver injury in experimental mice.(13)

Another study by Maryam M et al 2012 demonstrated that Nerium oleander Nerium oleander has strong antioxidant properties, including the ability to reduce and scavenge free radicals. In several test methods, distinct solvent extracts from Nerium oleander leaves and flowers demonstrated varying degrees of antioxidant activity in a concentration-dependent manner.(18) The quantity of total phenolic content contained in the individual extracts in each experiment was associated with the antioxidant activity. The most effective solvents for extracting antioxidants from Nerium oleander flowers and leaves were found to be methanol and aqueous methanol. The

associated extract had the greatest phenolic content of all the tests utilized here and also demonstrated the maximum antioxidant ability. These results are helpful for additional study to detect, isolate, and describe the particular chemical that causes (14)(19)

CONCLUSION

In conclusion after analyzing the percentage inhibition of Xanthine oxidase activity, the percentage inhibition of acetylcholinesterase and the percentage aggregation of amyloid beta plaque formation it was evident that the hydroalcoholic flower extract of nerium oleander plant possess neuroprotective potential to an extent and may help in curbing neurodegenerative diseases.

FUTURE SCOPE:

InVivo studies can be conducted and if the extract's neuroprotective potential is further validated, research can be directed toward developing safe and effective formulations, such as standardized extracts, nanoparticles, or encapsulated forms. Formulation development can enhance the extract's stability, bioavailability, and targeted delivery.

LIMITATIONS:

The cytotoxicity of the plant was not assessed in this study, assessing the cytotoxic effects assures safety . Therefore future research must be conducted to check the cytotoxic effects of the plant

AUTHOR CONTRIBUTIONS:

Katheeja Rilah S, Dr. Abirami Arthanari, Dr. Parameshwari

CONFLICT OF INTEREST:

None

ETHICAL CLEARANCE:

Not necessary for this study.

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