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Exploring The Potential Of Myricetin Against Free Radicals Formation And Neurodegeneration Using *In Vitro* Methods

[1]Darshinedevi Thirukumaran, [2]Lakshmi Thangavelu,

[2]*Royapuram Parthasarathy Parameswari

[1]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamilnadu, India.

[2]Centre for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Thandalam, Chennai, Tamilnadu, India.

***Corresponding author** Email: paramsarathy@gmail.com; parameswarirp.smc@saveetha.com

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ABSTRACT

Background: Oxidative stress and neuronal damage are common factors in the development of neurodegenerative disorders. Myricetin is a flavonoid compound found in various plant-based foods, such as berries, onions, and tea. It possesses potent antioxidant properties due to its ability to scavenge free radicals and inhibit oxidative processes. Myricetin can directly neutralize ROS and enhance the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). By reducing oxidative stress, myricetin may help protect neurons from damage in Alzheimer's disease. In view of the given background, the present study has investigated the effect of Myricetin on free radical products formation, in vitro acetylcholinesterase activity and amyloid beta peptide formation.

Materials and methods: The study has utilized in vitro enzyme inhibitory assay system to evaluate the antioxidant and neuroprotective potential of Myricetin. The DPPH assay assessed the free radical scavenging activity of the Myricetin. Further, the ABTS radical scavenging activity of Myricetin was determined. Xanthine oxidase inhibitory activity was assessed spectrophotometrically. Ascorbic acid was used as standard to compare the antioxidant effect of Myricetin. The inhibitory potential of Myricetin on *in vitro* acetylcholinesterase (AchE) activity and amyloid peptide aggregation were evaluated at various concentrations and compared with standard Donepezil hydrochloride.

Results: Myricetin exhibited significant antioxidant properties, reduces Aβ aggregation thereby attenuates neuroinflammation. These characteristics make myricetin a promising compound for targeting oxidative stress and neurodegeneration in Alzheimer's disease. However, more research is required to fully understand its therapeutic potential and translate these findings into effective treatments

Conclusion: In summary, the present study findings have demonstrated that myricetin can attenuate amyloid-beta (Aβ) aggregation, which is a hallmark pathological feature of AD. Myricetin has been shown to inhibit the formation of Aβ fibrils as well as inhibit AChE activity. These effects are crucial for preventing the formation of amyloid plaques and preserving neuronal function.

Keywords: Alzheimer disease, neuroprotection, Myricetin, antioxidant, flavonoid

INTRODUCTION

In recent years, there has been growing interest in the potential of natural compounds as therapeutic agents for combating oxidative stress and neurodegenerative disorders (1). Among these compounds, myricetin, a flavonoid found abundantly in various plant sources, has gained significant attention due to its potent antioxidant properties (1, 2). Oxidative stress caused by free radicals has been implicated in the pathogenesis of numerous neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Hence, exploring the potential of myricetin against free radical formation and neurodegeneration has

emerged as a promising area of research. Neurodegeneration, marked by the progressive loss of structure or function of neurons, lies at the heart of debilitating conditions like Alzheimer's disease (3). The growing prevalence of such disorders and the limitations of existing therapeutic interventions necessitate a continuous search for novel compounds with neuroprotective attributes (4, 5). Myricetin, with its inherent antioxidant properties, beckons attention due to its purported ability to counteract oxidative stress, a pivotal player in neurodegenerative processes.

Myricetin possesses a unique chemical structure that enables it to scavenge free radicals and mitigate oxidative damage in the body (5). Through its strong antioxidant activity, myricetin neutralizes reactive oxygen species (ROS) and prevents their harmful effects on cellular components such as lipids, proteins, and DNA. This property makes myricetin an attractive candidate for interventions aimed at reducing oxidative stress-induced neurodegeneration (6,7).

Studies have demonstrated the ability of myricetin to inhibit the generation of free radicals and to enhance the activity of endogenous antioxidant defense mechanisms. By modulating key enzymes involved in the production and detoxification of ROS, myricetin helps maintain redox homeostasis and protects neurons from oxidative damage. Additionally, myricetin exhibits anti-inflammatory effects, further contributing to its neuroprotective potential (6-8). Chronic inflammation is closely associated with neurodegenerative processes, and myricetin's ability to suppress inflammatory pathways may help alleviate neuroinflammation and slow down disease progression. The crux of myricetin's allure lies in its capacity to quench free radicals, the molecular entities notorious for their role in oxidative stress and neurodegeneration. Myricetin achieves this by donating electrons to stabilize and neutralize these highly reactive species. In the context of neurobiology, where neurons are particularly vulnerable to oxidative damage, myricetin emerges as a potential guardian, intercepting and mitigating the detrimental impact of free radicals on neural structures (9). Furthermore, myricetin has shown promising effects on various molecular targets involved in neurodegenerative pathways. It has been found to inhibit the aggregation of amyloid-beta (Aβ) peptides, which are implicated in Alzheimer's disease, and attenuate the formation of alpha-synuclein aggregates, characteristic of Parkinson's disease. Moreover, myricetin exhibits neuroprotective properties by promoting neuronal survival, enhancing synaptic plasticity, and modulating neurotransmitter systems (8). These multifaceted mechanisms suggest that myricetin may exert a broad spectrum of beneficial effects against neurodegeneration.

This inhibition of Aβ aggregation by myricetin has far-reaching implications for neuroinflammation, a cascading consequence of Aβ accumulation in the brain. Neuroinflammation, marked by the activation of glial cells and the release of pro-inflammatory mediators, contributes significantly to the progression of neurodegeneration. Myricetin's ability to attenuate neuroinflammation further solidifies its potential as a multifaceted therapeutic agent, addressing not only the upstream oxidative stress but also the downstream inflammatory responses intricately linked to neurodegenerative cascades (10). Given the increasing prevalence of neurodegenerative disorders and the limitations of current therapeutic approaches, there is a pressing need for novel strategies to prevent or delay disease progression. Exploring the potential of myricetin as a natural compound with antioxidant, anti-inflammatory, and neuroprotective properties holds great promise in this regard. By targeting free radicals and oxidative stress, myricetin may offer a viable avenue for the development of preventive or adjunctive therapies for neurodegenerative diseases (11). Therefore, in the present study the free radical scavenging activity as well neuroprotective effect of myricetin was evaluated using *in vitro* enzyme inhibition assays.

MATERIAL AND METHODS

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl(DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Xanthine, acetylthiocholine iodide, acetylcholine enzyme (0.3U/ml) were procured from Sigma-aldrich, USA.Ascorbic acid 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.

In vitro antioxidant activity

DPPH Free Radical Scavenging activity assay (12)

The 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay was performed to evaluate the free radical scavenging activity of the extract following previously described methods 0.004% DPPH solution was prepared in 100 ml of methanol. 10µL of myricetin at a concentration range of 10µM, 20µM, 40µM, 80µM, 160µM & 320µM was added to 190µL of DPPH solution. After vortexing, the mixture was incubated for 20 minutes at 37°C. The control blank contains solvent without the

test compound/standard. The decrease in absorbance of the test mixture (due to quenching of DPPH free radicals) was measured at 517 nm. The IC_{50} value was determined as the concentration of the test mixture that gave 50% reduction in the absorbance from a control blank. The experiments were repeated in triplicates and the percentage inhibition was calculated. Ascorbic acid was used as a reference standard. The DPPH radical scavenging activity of the extract was calculated by the below equation.

% scavenging effect = (Absorbance of control–Absorbance of sample) × 100/Absorbance of control.

ABTS radical scavenging assay (13)

ABTS radical scavenging activity was determined 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was dissolved in water and made to 7mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution was diluted with water to an absorbance of 0.70 (±0.02) at 734 nm. The reaction mixture consisted of 0.07mL of the myricetin at different concentrations (5-160µM) and 3mL of the ABTS radical. After incubation for 6 min, absorbance was determined in a spectrophotometer at 734 nm. Ascorbic acid was used as a reference standard. The antioxidant activity was calculated by using the following equation.

$$\% \text{ inhibition} = [(\text{Control-Test})/\text{Control}]*100$$

$A_{control}$ = Absorbance of negative control at the moment of solution preparation

A_{sample} = Absorbance of sample after 6 min

Xanthine oxidase inhibitory activity (14)

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions. The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50µL of different concentrations myricetin (10-320µM), different concentrations of Ascorbic acid (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 25ºC, 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 myricetin and Ascorbic acid. IC_{50} values were calculated from the mean values of data from three determinations. Ascorbic acid was used as reference standard.

***In vitro* acetylcholinesterase (AChE) inhibition assay (15)**

The myricetin 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 of myricetin and standard Donepezil hydrochloride were prepared using 0.05M tris base. Briefly, in this method, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of myricetin and Donepezil 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 (16). 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. Aβ solution (8µL) was mixed with the different concentrations of Myricetin (10-320µM) 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

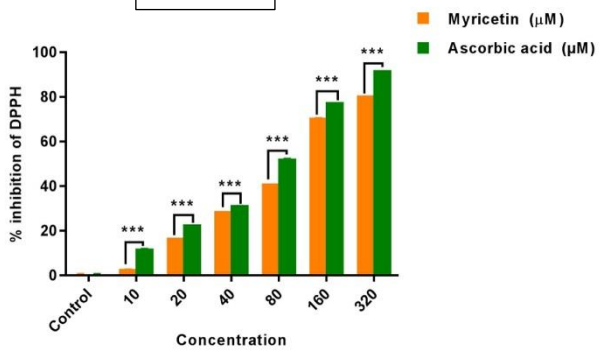
Data were analyzed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC_{50} values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standards groups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

RESULTS

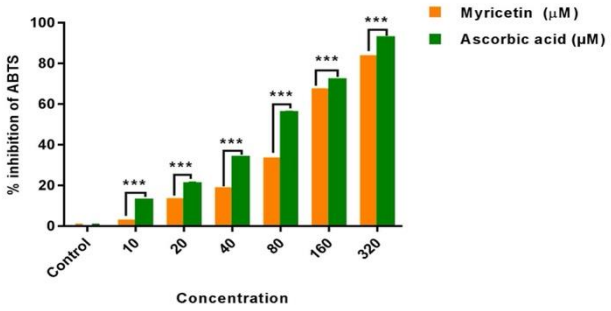
In vitro free radical scavenging activity of Myricetin

The free radical scavenging activity of Myricetin was evaluated using the DPPH radical scavenging assay, ABTS radical scavenging assay, and xanthine oxidase inhibitory assay. The results showed that Myricetin significantly ($p<0.001$) inhibited free radical formation at the highest tested concentration of 320µM. All three assays demonstrated inhibition percentage between 80-83% inhibition at the highest concentration of 320µM, compared to the standard ascorbic acid, which exhibited over 90% inhibition at a similar concentration. Additionally, Myricetin's percentage inhibition of free radical formation was found to be concentration-dependent.

DPPH



ABTS



Xanthine oxidase

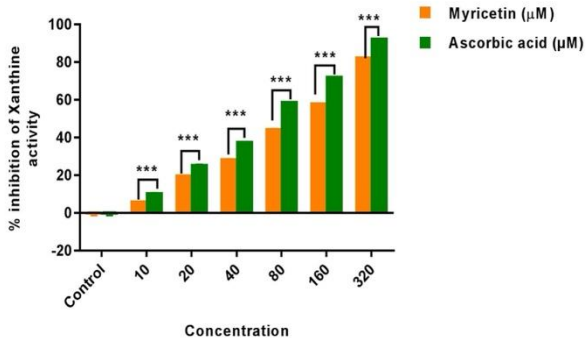


Figure 1: Free radicals scavenging effect of Myricetin at different concentrations compared with Standard Ascorbic acid. Data are expressed as Mean±SEM of triplicate values. Two-way ANOVA was used to assess differences between means at ***p<0.001, **p<0.01 and *p<0.05 level of significance vs Standard ascorbic acid. The means were compared with standards group using Holm-Sidak Test.

***In vitro* acetylcholinesterase activity**

To evaluate the effect of Myricetin on acetylcholinesterase activity, various concentrations (10-320µM) of Myricetin and the standard Donepezil hydrochloride were tested in combination with acetylthiocholine iodide, DTNB, and acetylcholinesterase enzyme. Both Myricetin and Donepezil showed significant inhibition of acetylcholinesterase activity across all tested concentrations (Figure 2). However, Myricetin demonstrated notably lower inhibitory activity compared to Donepezil at concentrations between 40-320µM (***p<0.001). The inhibition range for Myricetin was between 1.75% and 75.62% from lower to higher concentration whereas Donepezil's inhibition ranged from 6.73% to 91%.

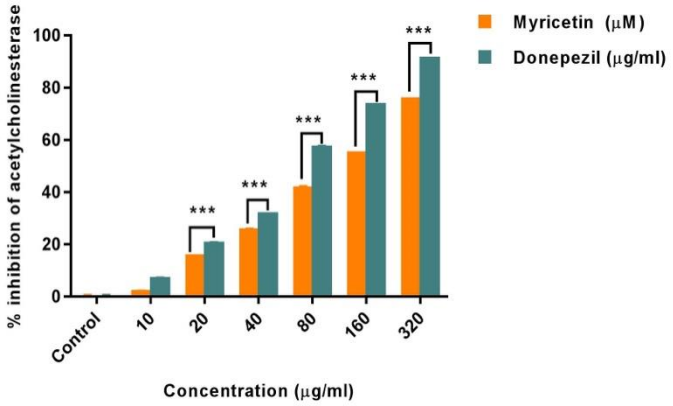


Figure 2: In vitro acetylcholinesterase inhibitory effect of Myricetin at different concentrations compared with Standard Donepezil hydrochloride. Data are expressed as Mean±SEM of triplicate values. Two-way ANOVA was used to assess differences between means at ***p<0.001, **p<0.01 and *p<0.05 level of significance vs Standard ascorbic acid. The means were compared with standards group using Holm-Sidak Test.

Effect of Myricetin on Amyloid (Aβ 1-42) Peptide Aggregation

The evaluation of Myricetin's effect on Aβ (1-42) aggregation demonstrated a significant inhibition compared to the standard Donepezil. At a concentration of 320µM, Myricetin markedly reduced Aβ (1-42) aggregation by 22.72%, while Donepezil, at 320µg/ml, achieved a reduction of 12.23%. This highlights a considerable decrease in amyloid aggregation due to Myricetin. Across the concentration range of 5 to 320µM, there was a significant difference (***p<0.001) in the inhibitory activity between Myricetin. Nevertheless, Myricetin's effect on inhibiting amyloid aggregate formation was similar to that of Donepezil.

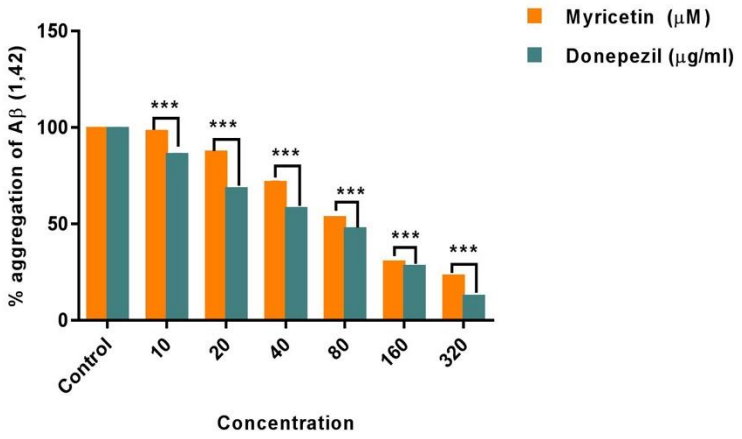


Figure 3: In vitro amyloid Aβ (1,42) inhibitory effect of Myricetin at different concentrations compared with Standard Donepezil hydrochloride. Data are expressed as Mean±SEM of triplicate values. Two-way ANOVA was used to assess differences between means at ***p<0.001, **p<0.01 and *p<0.05 level of significance vs Standard ascorbic acid. The means were compared with standards group using Holm-Sidak Test.

DISCUSSION

Neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, present a growing public health concern due to their increasing

prevalence and the limitations of current therapeutic approaches. Oxidative stress, stemming from the excessive production of reactive oxygen species (ROS) and free radicals, has been implicated in the pathogenesis of these disorders (5, 17). Hence, there is a pressing need for novel strategies that target oxidative stress and provide neuroprotection. In this context, the study explores the potential of myricetin, a natural compound found abundantly in various plant sources, as a therapeutic agent for combating neurodegeneration.

Myricetin's appeal as a candidate for neuroprotection stems from its potent antioxidant properties. The unique chemical structure of myricetin equips it with the ability to effectively scavenge free radicals and mitigate oxidative damage in the body (18). The process involves neutralizing ROS, thus preventing their detrimental effects on essential cellular components such as lipids, proteins, and DNA (19). This property positions myricetin as an attractive candidate for interventions aimed at reducing oxidative stress-induced neurodegeneration. The study employs *in vitro* antioxidant assays to investigate myricetin's efficacy in combating oxidative stress. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assays, along with the xanthine oxidase inhibitory activity assay, provide valuable insights into myricetin's antioxidant capabilities (12, 13). The findings indicate that myricetin is effective in scavenging free radicals and inhibiting xanthine oxidase. These results align with the broader literature on myricetin, which underscores its potential as an antioxidant and its capacity to counteract oxidative stress.

In Alzheimer's disease (AD), a common feature is the presence of acetylcholinesterase (AChE), which is often associated with β-amyloid plaques and neurofibrillary tangles (NFT). AChE appears to directly interact with amyloid-β, possibly promoting the aggregation of this peptide into insoluble plaques. This newly discovered role suggests that AChE inhibitors could act as disease-modifying agents (20). Myricetin's role in inhibiting the acetylcholinesterase activity and influencing Aβ (amyloid-beta) concentration is another significant aspect of this study. Aβ aggregation is a central feature of Alzheimer's pathology, leading to the formation of amyloid plaques, which are implicated in neurodegeneration (21). Myricetin significantly inhibited the acetylcholinesterase activity as well as amyloid protein aggregation, suggesting its potential role in modulating this crucial aspect of Alzheimer’s disease. This finding adds to the appeal of myricetin as a multi-faceted candidate for neuroprotection.

In the context of current therapeutic approaches for neurodegenerative disorders, the limitations and challenges are notable. While some medications offer symptomatic relief, there is a lack of disease-modifying treatments that can halt or slow down disease progression (22). This underscores the significance of exploring natural compounds like myricetin, which offer the potential for a multi-pronged approach to neuroprotection. Myricetin's antioxidant properties and potential influence on Aβ aggregation provide a valuable foundation for further research in this field. This study sheds light on the potential of myricetin as a therapeutic agent for neurodegenerative disorders. Its robust antioxidant properties, as demonstrated through in vitro assays, make it a promising candidate for further research and potential clinical applications (23). Further research, both in preclinical and clinical settings, is warranted to explore the full potential of myricetin in preserving brain health and combating neurodegeneration.

CONCLUSION

To summarize, myricetin showcases notable antioxidant attributes and effectively mitigates neuroinflammation by diminishing Aβ aggregation. These distinctive characteristics mark myricetin as a compound with considerable promise in targeting both oxidative stress and neurodegeneration in the context of Alzheimer's disease. The potential therapeutic utility of myricetin is underscored by its dual action in reducing oxidative stress and attenuating the aggregation of Aβ proteins, a hallmark feature in Alzheimer's pathology. However, despite these encouraging findings, a more extensive and in-depth research endeavor is imperative. This is essential not only for a comprehensive understanding of myricetin's therapeutic potential but also to facilitate the translation of these research insights into practical and effective treatments for Alzheimer's disease. The journey from promising compound to clinically effective treatment necessitates further exploration and validation of myricetin's efficacy, emphasizing the ongoing need for rigorous scientific inquiry in the realm of neurodegenerative disorders.

CONFLICT OF INTEREST

There is no conflict of interest.

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