Research Article

Drug delivery of neurotransmitters by nanodrugs synthesized from mangiferin

T. K. Hariprasath, V. Vishnu Priya*, R. Gayathri, J. Selvaraj

ABSTRACT

Background: Cost-effectiveness and synthesis of metallic nanoparticles are a fast-growing research in nanotechnology. Mangiferin is a famous biologically active phenolic compound that is present in large amounts in the leaf of *Mangifera indica*. **Aim:** The study was aimed at assessing the evaluation of neuroprotective potential of silver nanoparticles (AgNPs) synthesized from mangiferin. **Materials and Methods:** To evaluate the neuroprotective effect, SHSY5Y cells were treated with different concentrations of mangiferin AgNPs (2.5–20 µg/ml) for 4 h and then incubated with 1-methyl-4-phenylpyridinium (MPP^+) for 2 h. The effective dose of AgNPs was used to identify potential neuroprotective effects against MPP^+ toxicity by acetylcholinesterase inhibitory activity. **Results:** Mangiferin AgNPs showed a significant increase in the acetylcholine esterase inhibitory activity in a dose-dependent manner (2.5, 5, 10, and 20 µg/ml), and the inhibitory activity was found to be 24.5%, 44.6%, 52.1%, and 70.8%, respectively. IC_{50} value of the nanodrugs was found to be 9.59 (µg/ml). **Conclusion:** This present clearly shows that mangiferin AgNPs have potential neuroprotective efficacy, and hence, it can be used as one of the therapeutic neuroprotective drugs.

KEY WORDS: Mangiferin, Neuroprotective activity, SHSY5Y cells, Silver nanoparticles

INTRODUCTION

Mangiferin is a plant natural polyphenol of C-glycosylxanthone structure and various pharmacological activities. It can be found in many plant species, among which the mango tree (*Mangifera indica*) is one of the primary sources. It is also present in some medicinal herbs, influencing their therapeutic and preventive properties, and in honeybush (*Cyclopia* sp.), a popular South African herbal tea.[1] Mangiferin dissolves well in water, so it can be easily extracted into infusions and decoctions. In the mangiferin molecule, four aromatic hydroxyl groups determine its strong antiradical and antioxidant properties. Numerous published *in vitro* and *in vivo* pharmacological studies demonstrated many other activities of mangiferin: Analgesic, antidiabetic, antisclerotic, antimicrobial, and antiviral, cardio-, hepato-, and neuro-protective, anti-inflammatory, antiallergic, monoamine oxidase inhibiting, and memory improving, as well as radioprotective against X-ray, gamma, and ultraviolet (UV) radiation. Several studies indicated also its ability to inhibit cancerogenesis and cancer cells growth by apoptosis induction *in vitro* and *in vivo*.[1]

Nanotechnology is the process of synthesizing nanoparticles of variable sizes (1–100 nm), shapes, and chemical compositions with controlled dispersity for human benefits. This technology offers nanocomposites or nanostructures in new products by technological processes. In addition, it is significant on account of its pre-eminence in the medical field for its extensive applications.[2] Even in other fields such as pharmacy and food production or packaging, researchers on nanoparticles are increased as they are effectively a bride between bulk materials and atomic or molecular structures.[3,4] Plant compounds for metallic nanoparticles preparation are the most common method utilized to enhance its application in medicine.[5]

Among various metals used for nanoparticles production, silver nanoparticles (AgNPs) are considered to be of great importance in the medical field as they can be synthesized by a chemical

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method or by biological method. Indeed, silver has highly specific surface area for its maximum contact with environment and has strong antimicrobial property.[6] When comparing the noble metals, silver is also the widely used metal due to its unique properties as wound healer agent its use in the biomedical field. Studies have shown that AgNP synthesed from plant extracts or the plant compounds with perfect structural properties are more effective.[7] When comparing the noble metals, silver is also the widely used metal due to its unique properties as wound healer agent in the biomedical field. When comparing the various methods such as physical, chemical, enzymatic, and biological methods that are involved in the synthesis of AgNPs, biological methods using the plant extracts have been gained considerable interest due to the use of environmentally benign materials.[8] Although there are numerous studies on the pharmacological properties of *M. indica,* neuroprotective effect of mangiferin AgNP has not been explored so far. Hence, in this study, we have shown the potential neuroprotective effect mangiferin AgNPs in neuronal cells (SHSY5Y) *in vitro.*

MATERIALS AND METHODS

Synthesis of AgNPs from Mangiferin

The aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of AgNPs. 10 ml of mangiferin dissolved in dimethyl sulfoxide (DMSO) was added into 90 ml of aqueous solution of 1 mM $AgNO_3$ for reduction into Ag^+ ions and kept for incubation period of 15 h at room temperature. From the stock solution of AgNPs, different concentrations (2.5–20 µg/ml) using the media were prepared.[9,10]

Cell Culture and Maintenance

SHSY5Y cells were cultured in minimal essential medium with Earl's salts (EMEM) supplemented with 10 % fetal bovine serum, 1% non-essential amino acids, 2 mM L-glutamine, and 1% PEST. The cells were plated at a density of 500 cells/mm^2 in 96-well plate overnight in the culture medium. The medium was replaced with the differentiation medium (Dulbecco's modified medium with Ham's F12 medium [1:1], 1% N_2 supplement, and 1% PEST) containing 1 µM RA. The cells were differentiated for 3–6 days. Half of the medium per well was changed every 48 h.

Cells Treatment

The cells were pretreated with different concentrations of AgNPs from mangiferin (2.5–20 µg/ml) for 4 h and then incubated with 1-methyl-4-phenylpyridinium (MPP^+) (1 mM) for 2 h. The effective dose of AgNPs was used to identify potential neuroprotective effects against MPP^+ toxicity.

Assessment of Acetylcholinesterase (AChE) Inhibitory Activity

In this study, AChE inhibitory activity in SHSY5Y cells was determined by the method of Ellman *et al.* (1965). Briefly, different concentrations of the sample were incubated with 10µL of AChE for 45min at room temperature. To the reaction mixtures, 125µL of 3mM 2-nitrobenzoic acid was added and the total volume was made up to 1mL with Tris-HCl buffer (pH 8.0). Subsequently, 25µL of 15mM acetylthiocholine iodide (AThCh) was added to the reaction mixtures to initiate the enzyme activity. The formation of 5-thio-2-nitrobenzoate anion was detected by yellow coloration and the absorbance was detected in the wavelength of 405nm using UV-visible spectrophotometer. The experiments were done in triplicates. In this study, donepezil used standard.

Percentage of inhibition = ([Enzyme activity without sample−enzyme activity with sample]/Enzyme activity without sample) × 100.

Statistical Analysis

Results were expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA) and *post hoc* least significant difference test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of Mangiferin AgNPs on AChE Inhibition in SHSY5Y Cells

Table 1 represents the inhibition of acetylcholine esterase by mangiferin coated AgNPs in SHSY5Y cells and compared with standard drug donepezil. Mangiferin AgNPs significantly increased ($P < 0.05$) the AChE inhibitory activity in a dose-dependent manner (2.5, 5, 10, and 20 µg/ml), and the inhibitory activity was found to be 24.5%, 44.6%, 52.1%, and 70.8%, respectively. IC_{50} value of the nanodrugs was found to be 9.59 µg/ml. The percentage of inhibitory activity of standard drug donepezil was found to be 82.6%. This study shows that mangiferin AgNPs could effectively show neuroprotective effect near to that of the commercially available standard drug donepezil [Table 1].

DISCUSSION

Nanoparticles have been submerged in a variety of medical and commercial products such as cosmetics, bioanalytics, optoelectronics, biomedicines, and antibacterials, due to their inimitable physical, chemical, and biological properties.[11-13] However, among different nanoparticles, AgNPs are more widespread in industrial and consumer applications and have been quite rigorously studied.

Table 1: Effect of mangiferin AgNPs on acetylcholinesterase inhibition in SHSY5Y cells in comparison with standard drug donepezil

Treatment	Concentration (µg/ml)	Abs 405 nm Mean±SD	% of inhibition
SHSY5Y untreated cells	-	0.374±0.17	
AgNPs	2.5	0.282±0.15*	24.5
	5.0	0.207±0.20*	44.6
	10	0.179±0.19*	52.1
	20	0.109±0.09*	70.8
Donepezil (µM)	1.5	0.065±0.03*	82.6

Each value represents as mean±standard deviation (n=3) of three independent observations. *P<0.05 significantly different as compared with SH5YHY control. AgNPs: Silver nanoparticles

Nanoparticles synthesized from herbal plant extracts were considered safer than chemically synthesized. However, in this study, we have explored the effect of green synthesized AgNPs against AChE to suggest their protective potential against neurological disorder. Here, AgNPs was synthesized by utilizing the mangiferin dissolved in DMSO. The synthesized AgNPs were characterized duly by UV–visible spectrophotometry, scanning electron microscopy, and transmission electron microscope (results were not shown here). The objective of the present work was to analyze the effect of herbally synthesized AgNPs against AChE activity. AgNPs exhibited a noteworthy "concentration-dependent inhibition" of AchE, whereby ATCI was used as the substrate.

Šinko *et al.*[14] conducted a study to observe the effect of chemically synthesized AgNPs against AChE activity. They found that hydroxylamine hydrochloride synthesized AgNPs were more potent inhibitors of AChE with an IC_{50} value of 49 ± 1 µmol dm^{-3} among different chemically synthesized AgNPs. In our present study, we observed significantly increased (P < 0.05) the AChE inhibitory activity in a dose-dependent manner (2.5, 5, 10, and 20 µg/ml) and the inhibitory activity was found to be 24.5%, 44.6%, 52.1%, and 70.8%, respectively. IC_{50} value of the nanodrugs was found to be 9.59 µg/ml. Our study confirms that AgNPs synthesized from mangiferin were significant inhibitor of AChE even at the lowest concentration, i.e., 2.5 µg/ml. The maximum concentration we used for the present study was 20 µg/ml of AgNPs showed almost 70.8% inhibition of AChE activity.

CONCLUSION

The biologically synthesized nanoparticles are generally considered safer for use. In contrast, in this research piece, we found that AgNP synthesized by mangiferin showed potent inhibitory potential against an important neurological enzyme, i.e., AChE. Hence, it is concluded from the present findings that mangiferin AgNPs have potential neuroprotective efficacy and, it can be used as one of the therapeutic neuroprotective drugs. Further studies on *in vivo* model are warranted to show ascertain the mangiferin AgNPs.

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