



# Neuroprotective potential of the oxindole alkaloids isomitraphylline and mitraphylline in human neuroblastoma SH-SY5Y cells

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## Abstract

The purified oxindole alkaloids, isomitraphylline and mitraphylline from *Uncaria perrottetii*, revealed their ability to break amyloid aggregates in vitro suggesting their therapeutic potentials in Alzheimer's disease (AD). Thioflavin-T assay for assessing amyloid- $\beta$  ( $A\beta$ ) aggregation of these alkaloids exhibited inhibitions at  $60.321\% \pm 2.61$  (50  $\mu$ M) for isomitraphylline and  $43.17\% \pm 3.48$  (50  $\mu$ M) for mitraphylline. Neuroprotective effects were elaborated against  $A\beta$ -induced SH-SY5Y cells at 20  $\mu$ M and 10  $\mu$ M for isomitraphylline, and 20  $\mu$ M for mitraphylline. In addition, both alkaloids attenuated and protected the  $H_2O_2$ -induced SH-SY5Y cell cytotoxicity at 20  $\mu$ M. The intracellular ROS levels of SH-SY5Y cells from  $H_2O_2$ -induced oxidative stress were reduced at 20  $\mu$ M and 10  $\mu$ M, and the mitochondrial membrane potentials of  $A\beta$ -induced SH-SY5Y cells were protected at 20  $\mu$ M. The overall results suggested the potentials of both alkaloids to target certain pathological biomarkers of AD and could be further investigated as therapeutic or preventive drug leads against AD.

**Keywords** Alzheimer's disease · Oxindole alkaloids · SH-SY5Y cells · *Uncaria*

## Introduction

Alzheimer's disease (AD) is a dominant neurological disorder characterized by cognitive impairments and synaptic dysfunctions affecting mostly the elderlies (Bagyinzsky et al. 2017). Similar with other neurological diseases, the progression of AD is correlated to mitochondrial dysfunction, oxidative stress, protein misfolding, abnormal amyloid- $\beta$  ( $A\beta$ ) deposition, alterations in calcium homeostasis, and inflammation (Taylor et al. 2016). It is estimated that about 82 million people in 2030 and 152 million in 2050 worldwide will be affected by AD (Emmerzaal et al. 2015). Currently, there are only five drugs approved by the US FDA to minimize the progression of symptoms related to

AD. These are natural product-based compounds, which include the acetylcholinesterase inhibitors, galantamine, revastigmine, donepezil, and tacrine, and the NMDA receptor antagonist memantine (Guo et al. 2016; Huang et al. 2019). These drugs only provide symptomatic relief of AD without significant effectiveness in the disease progression and prevention. Hence, the challenges of finding an alternative treatment or therapy from natural products are warranted. Due to their diverse structures and pharmacological activities, natural products and their derivatives continuously play an important role in the discovery and drug development as potential avenues of finding therapeutic agents in neurodegenerative diseases (Angeloni and Vauzour 2019).

The genus *Uncaria* Schreb. (Rubiaceae) comprised of 34 species and distributed among tropical areas in Africa, Southeast Asia, and South America (Ridsdale 1978). In the Philippines, ten species were reported, including the two endemic *U. nervosa* Elmer and *U. perrottetii* (A. Rich.) Merr. Several compounds were elaborated from phytochemical studies of *Uncaria* species containing alkaloids, flavonoids, phenylpropanoids, quinovic acid glycosides, and triterpenoids, in which the alkaloids were identified predominantly as bioactive constituents (Heitzman et al. 2005). Pharmacological investigations revealed antiviral, anti-inflammatory, antioxidant, cytotoxicity, immune-stimulant,

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hypotensive, mutagenicity, and antibacterial properties (Heitzman et al. 2005). The potent in vivo inhibitory activity of the extracts of *U. rhynchophylla* against AD and Parkinson's disease were previously reported (Shim et al. 2009; Xian et al. 2011). As part of our research discovery in finding neuroprotective agents from nature and their potential mechanisms, two isomeric oxindole alkaloids, isomitraphylline and mitraphylline, were purified from the Philippine endemic *U. perrottetii* and their protection and/or cytotoxicity in oxidative stress-induced human neuroblastoma SH-SY5Y cells were investigated. The potential neuroprotective effects of isomitraphylline and mitraphylline against amyloid aggregation were not investigated previously.

## Materials and methods

### General considerations

The extraction of *U. perrottetii* leaves and the isolation and identification of the alkaloids from the *U. perrottetii* crude base extract have been previously reported (Olivar et al. 2018). Isomitraphylline (45 mg) and mitraphylline (38 mg) were the predominant alkaloids isolated from the crude base extract based on the afforded weights. The NMR spectra of the alkaloids are presented in the Online Resource Material. A $\beta$ <sub>1-42</sub> (Aggresure™) was dissolved at 250  $\mu$ g/mL in sterile PBS.

### Thioflavin T (ThT) assay

The inhibition of A $\beta$ <sub>1-42</sub> aggregation was evaluated as previously described (Tan et al. 2019; Xia et al. 2019). Briefly, A $\beta$  dissolved in PBS was incubated with or without the alkaloids or phenol red (positive control) at 37 °C for 24 h in a 384-well plate. ThT solution was added and incubated for another 15 min. Fluorescence signal (Ex 450 nm; Em 510 nm) was measured using a PerkinElmer Victor-3® multi-plate reader.

### Cell culture

Human neuroblastoma SH-SY5Y cells (ATCC CRL-2266) were maintained in DMEM supplemented with 10% FBS, 1% kanamycin, and 1% penicillin at 37 °C and 5% CO<sub>2</sub> and passaged twice per week. Experiments were performed at 80–90% confluence.

### Cell Cytotoxicity

Cell viability measurements were performed using the ATP luminescence assay as described previously (Tan et al. 2019, 2020a). SH-SY5Y cells at  $2 \times 10^4$  cells/well density were

subcultured in 96-well plate and incubated for 24 h. After incubation, cells were treated with the alkaloids for 24 h. The media were removed, cells were washed with PBS, fresh media were added, and incubated for another 30 min. Then, CellTiter-Glo® luminescent reagent was added and the luminescence was read on a multi-plate reader. Data were analyzed and the % cell viability was expressed relative to the control.

### Neuroprotective activity assay

Determination of the neuroprotective activity in A $\beta$  or H<sub>2</sub>O<sub>2</sub>-induced SH-SY5Y cytotoxicity was performed as previously described (Gonzalez-Sarrias et al. 2017; Yu et al. 2014a) and evaluated by the ATP luminescence assay. Neuroblastoma SH-SY5Y cells were seeded in 96-well plate at  $2 \times 10^4$  cells/well and incubated for 24 h. After stabilization, cells were pre-treated with the alkaloids for 6 h before incubation with A $\beta$ <sub>1-42</sub> (5  $\mu$ M) or H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M). A solvent control (untreated control cells), A $\beta$  or H<sub>2</sub>O<sub>2</sub> alone, and alkaloids alone treatments were also included. After incubation, the % cell viabilities were determined in triplicate experiments.

### Measurement of reactive oxygen species (ROS)

ROS measurements were performed using the 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCFDA) staining method as previously described (Gonzalez-Sarrias et al. 2016; Peñalver et al. 2020). After 24 h stabilization, SH-SY5Y ( $2 \times 10^4$  cells/wells) cells were pre-treated with the alkaloids for 2 h before incubation with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for another 4 h. After incubation, cells were treated with 25  $\mu$ M H<sub>2</sub>DCFDA and incubated for another 2 h in the dark at 37 °C. Fluorescence intensity (Ex 495 nm, Em 520 nm) was measured in a microplate reader. The ROS level was calculated as a percentage of the untreated control cells (100%) in triplicate measurements.

### Mitochondrial membrane potential ( $\Delta\Psi$ m) assay

Measurement of the  $\Delta\Psi$ m was performed using the tetramethylrhodamine, methyl ester (TMRE) staining method as previously described (Alvariño et al. 2019). After 24 h SH-SY5Y ( $2 \times 10^4$  cells/well) acclimatization, cells were pre-treated with the alkaloids for 2 h and then combined with 5  $\mu$ M A $\beta$ <sub>1-42</sub> for 24 h. After treatment, 1  $\mu$ M TMRE staining solution was added and incubated at 37 °C for 30 min. The fluorescence (Ex 549 nm, Em 575 nm) was read in a microplate reader. The  $\Delta\Psi$ m was calculated as a percentage of the untreated control cells (100%) in triplicate measurements.

## Statistical analysis

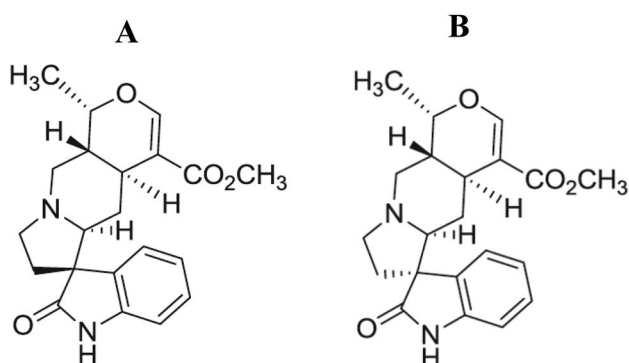
Data are reported as the mean  $\pm$  SD of at least three experiments. Statistical analysis was determined by one-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test. Statistical significance was considered at  $*p < 0.05$ .

## Results and discussion

In the continuous search for potential neuroprotective natural products against AD (Tan et al. 2019, 2020a, b), the alkaloids isomitraphylline and mitraphylline (Fig. 1) from previous purifications from *U. perrottetii* (Olivar et al. 2018) were investigated. These two alkaloids were abundant in the genus *Uncaria*. Isomitraphylline was isolated from 18 *Uncaria* species, and mitraphylline could be isolated from 20 *Uncaria* species (Olivar et al. 2018). Previous reports on these oxindole alkaloids demonstrated their cytotoxic and antiproliferative effects in various cancer cell lines including their potential as anticancer agents (Bacher et al. 2006; Pilariski et al. 2010; Yu et al. 2015). Furthermore, these alkaloids were the major compounds identified in the extracts of *U. tomentosa* by UPLC-MS analysis (Azevedo et al. 2019). The crude extract of *U. perrottetii* was shown to have an immunostimulatory effects in vivo (Nudo and Catap 2011). The study of the biological activity of isolated natural products from the plant extracts would be essential in elucidating their potential cellular and molecular mechanisms.

### Thioflavin T (ThT) assay

The abnormal aggregation of A $\beta$  peptides is one of the pathological characteristics of AD. This could lead to the formation of its oligomers, protofibrils, and insoluble plaques which would cause dysfunctions in mitochondrial, resulting



**Fig. 1** Chemical structure of isomitraphylline (a) and mitraphylline (b)

in increases of oxidative stresses and neuroinflammations (Jakob-Roetne and Jacobsen 2009). The inhibition of A $\beta$  aggregation of the two alkaloids was determined using the ThT assay. Both alkaloids showed moderate inhibitory effects of 61.32% ( $\pm 2.61$ ) and 63.27% ( $\pm 3.48$ ) at 50  $\mu$ M concentrations of isomitraphylline and mitraphylline, respectively. These results indicated a no significant difference ( $p < 0.05$ ) in comparison to the phenol red as the positive control (Necula et al. 2007; Wu et al. 2006) with 67.31% ( $\pm 3.04$ ) inhibition at 50  $\mu$ M. At 5  $\mu$ M, isomitraphylline and mitraphylline showed 21.63% ( $\pm 5.47$ ) and 19.96% ( $\pm 3.85$ ) inhibitions of A $\beta$  aggregation, respectively. The capability of the two alkaloids for breaking A $\beta$  aggregations prompted the investigations of their neuroprotective effects in oxidative stress-induced neuroblastoma SH-SY5Y cells from A $\beta_{1-42}$  and H $_2$ O $_2$ , and their underlying mechanisms were evaluated focusing on the ROS productions and mitochondrial membrane potentials.

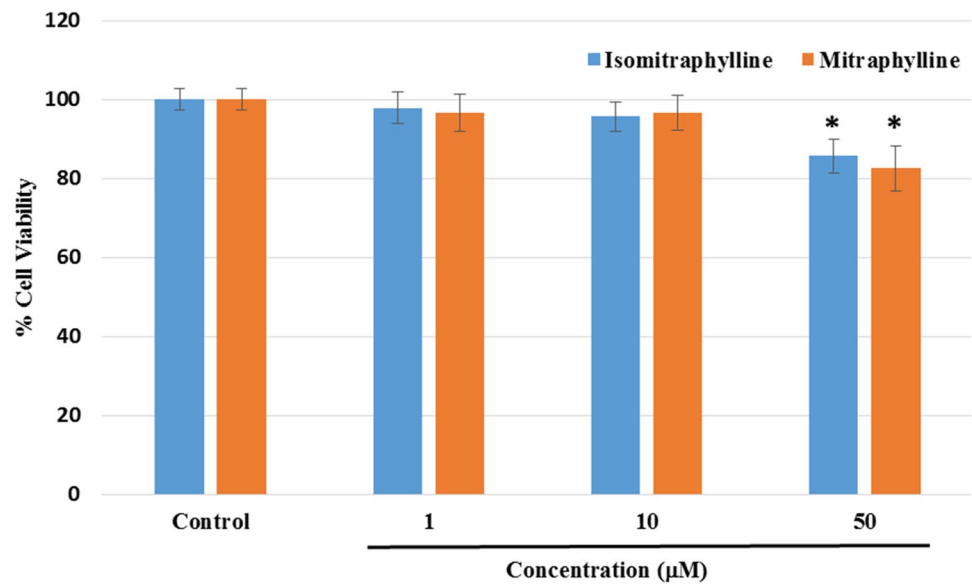
### Effect of the alkaloids on viability of SH-SY5Y cells

Before the neuroprotective experiments, the cytotoxicity in neuroblastoma SH-SY5Y cells of the alkaloids at 1, 10, and 50  $\mu$ M concentrations was determined by measuring the cell viability using the ATP luminescence assay. The cells were incubated with the compounds for 24 h. Figure 2 showed a significant difference in the % cell viability using the highest concentration at 50  $\mu$ M, while the 10 and 1  $\mu$ M did not exert any significant cytotoxic effect ( $p < 0.05$ ) in comparison to the control cells. Hence, the succeeding treatments for the neuroprotection assay used 1, 10, and 20  $\mu$ M concentrations.

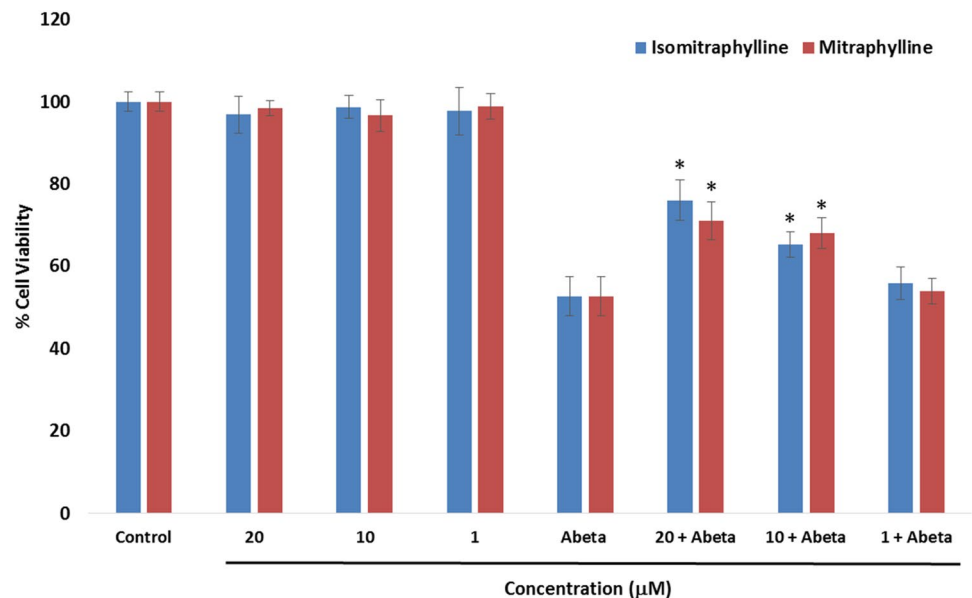
### Neuroprotective effects of the alkaloids on the oxidative stress-induced cytotoxicity

The ability of the alkaloids to protect human neuroblastoma SH-SY5Y cells against oxidative damage stimulated by A $\beta_{1-42}$  or H $_2$ O $_2$  was evaluated by the ATP luminescence assay. In Fig. 3, the alkaloids were tested for their neuroprotective potential against A $\beta$ -induced cell injuries in SH-SY5Y cells. A $\beta$  had been widely employed to generate neuronal cell damage to analyze the protective potential of plant extracts and natural products (An et al. 2019; Okello et al. 2011; Park and Kim, 2002; Yu et al. 2014b). A 5  $\mu$ M concentration, A $\beta$  revealed 52.71% cell viability. The neuroprotective effects were evaluated by pre-treatment of the cells with the alkaloids for 6 h before incubation with the 5  $\mu$ M A $\beta$  for 24 h. In the non-A $\beta$ -treated groups, the cells did not exert any antiproliferative activity against the SH-SY5Y cell line. In the A $\beta$ -treated groups, both the alkaloids at 10  $\mu$ M and 20  $\mu$ M showed significant protective effects ( $p < 0.05$ ) in comparison to the A $\beta$ -treated only cells. Isomitraphylline exhibited a cell viability of 65.32% ( $\pm 3.06$ ) at 10  $\mu$ M and

**Fig. 2** Effects of the alkaloids on cell viability in neuroblastoma SH-SY5Y cells as determined by the ATP luminescence assay. The results indicate % cell viability vs the control cells (untreated) and reported as mean  $\pm$  SD of triplicate experiments. The (\*) indicates a significant difference with the control cells at  $p < 0.05$



**Fig. 3** Neuroprotective effects of the alkaloids on  $A\beta_{1-42}$  (Abeta)-induced cytotoxicity using the ATP luminescence assay. Neuroblastoma SH-SY5Y cells were pre-treated with the alkaloids for 6 h, followed by treatment with 5  $\mu$ M  $A\beta$  for 24 h. The results indicate % cell viability vs the control cells and reported as mean  $\pm$  SD of triplicate experiments. The (\*) indicates a significant difference with the  $A\beta$ -treated group at  $p < 0.05$

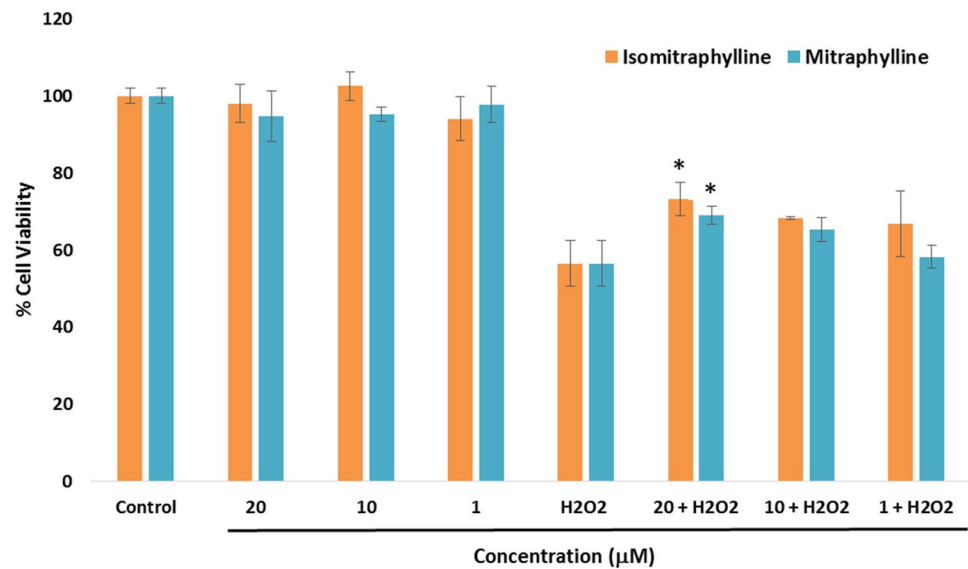


76.02% ( $\pm 4.89$ ) at 20  $\mu$ M. Mitrephylline revealed 68.04% ( $\pm 3.79$ ) and 71.06% ( $\pm 4.56$ ) cell viabilities at 10  $\mu$ M and 20  $\mu$ M, respectively. The 1  $\mu$ M alkaloid concentration did not show protective effect in comparison to the  $A\beta$ -treated group ( $p < 0.05$ ). These results justified the inhibition of  $A\beta$  aggregation of these alkaloids as suggested from the potential screening drug candidates by ThT assay. Furthermore, the neuroprotective effects of mitrephylline also corroborated with previous data demonstrating the significant binding of mitrephylline with the  $A\beta_{1-40}$  protein (Frackowiak et al. 2006).

The effects of the alkaloids on the oxidative damage caused by  $H_2O_2$  were evaluated as shown in Fig. 4.  $H_2O_2$  treatment (100  $\mu$ M) of the SH-SY5Y cells decreased

the cell viability to 56.45% in comparison to the control group, while the non- $H_2O_2$ -treated cells showed no significant cell viability difference ( $p < 0.05$ ). To determine the neuroprotective activity, the cells were pre-treated with various concentrations of the alkaloids for 6 h, followed by subsequent addition of the 100  $\mu$ M  $H_2O_2$  for 24 h. Both alkaloids provided significant neuroprotection to the neuroblastoma SH-SY5Y cells against  $H_2O_2$ -induced cytotoxicity at 20  $\mu$ M with cell viability of 73.14% ( $\pm 4.38$ ) for isomitrephylline and 69.03% ( $\pm 2.43$ ) for mitrephylline in comparison to the  $H_2O_2$ -treated cells ( $p < 0.05$ ). Both alkaloids did not show significant protection against  $H_2O_2$  to SH-SY5Y cells at lower concentrations (1 and 10  $\mu$ M).

**Fig. 4** Neuroprotective effects of the alkaloids on  $H_2O_2$ -induced SH-SY5Y cell cytotoxicity using the ATP luminescence assay. The cells were pre-treated with the alkaloids for 6 h, before incubation with  $100 \mu M H_2O_2$  for 24 h. The results indicate % cell viability vs the negative control and reported as mean  $\pm$  SD of triplicate experiments. The (\*) indicates a significant difference with the  $H_2O_2$ -treated group at  $p < 0.05$



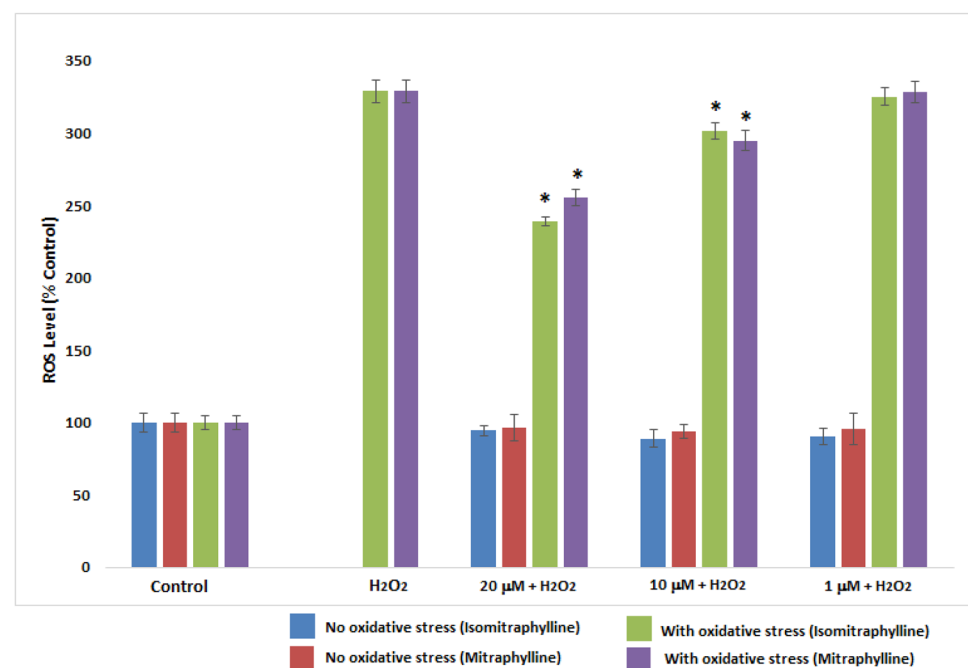
### Effects of the alkaloids on the ROS production and mitochondrial membrane potential

The increase of misfolded proteins contributed to mitochondrial dysfunction and proliferation of ROS releases in neuronal cells (Kumar et al. 2018). Microglial activation also could influence the elevated level of ROS and reactive nitrogen species in AD, resulting in oxidative damaged environment and cell death (Ali et al. 2013). Also, exposure of the neuroblastoma SH-SY5Y cells to various stressors led to superoxide productions (Chen et al. 2013). In fact, the free radicals could attack the membrane phospholipids

and damage DNA, causing loss of mitochondrial membrane potentials, mutated proteins, and cell apoptosis (Wang et al. 2010; Yu et al. 2014b; Zeng et al. 2010).

The effect of the alkaloids on the intracellular ROS levels was shown in Fig. 5. SH-SY5Y cells were pre-treated with the alkaloids in their non-cytotoxic concentrations for 2 h and incubated with  $100 \mu M H_2O_2$  for another 4 h to induce oxidative damage. The ROS levels generated were evaluated using 2',7'-dichlorodihydrofluorescein diacetate ( $H_2DCFDA$ ) staining. Cells treated with  $H_2O_2$  alone showed an enhanced intracellular ROS levels (329.06%) in comparison to the control cells. Both alkaloids significantly reduced

**Fig. 5** Effect of the alkaloids on the intracellular ROS level. SH-SY5Y cells were incubated with the alkaloids for 6 h (No oxidative stress). SH-SY5Y cells were pre-treated with the alkaloids for 2 h, and incubated with  $100 \mu M H_2O_2$  for another 4 h (With oxidative stress). ROS generation was measured using 2',7'-dichlorodihydrofluorescein diacetate ( $H_2DCFDA$ ). Values are the mean  $\pm$  SD of triplicate experiments and expressed as % of the control. The (\*) indicates a significant difference compared to the  $H_2O_2$ -treated group alone at  $p < 0.05$





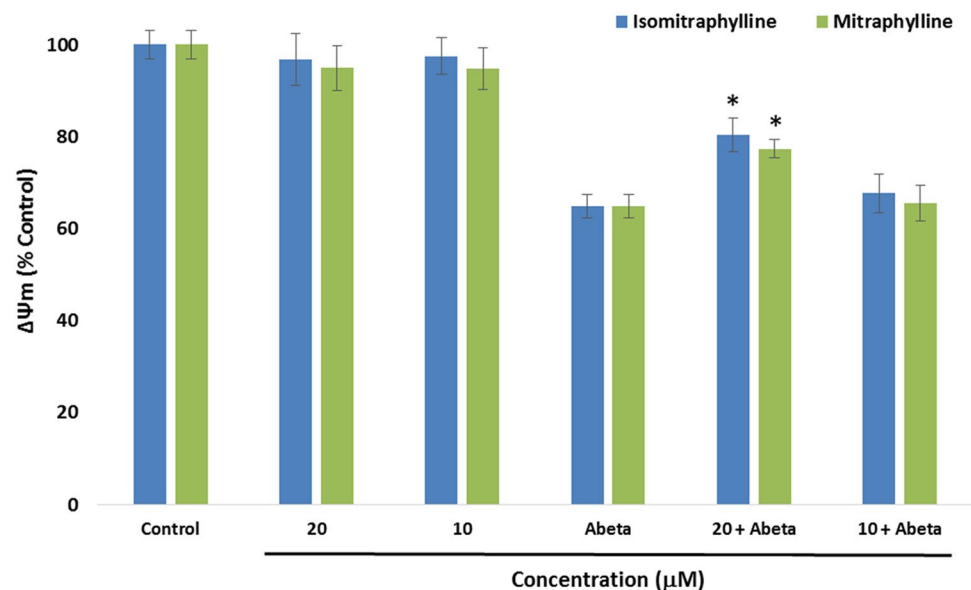
ROS levels at 10  $\mu\text{M}$  and 20  $\mu\text{M}$  concentrations in comparison to the  $\text{H}_2\text{O}_2$ -treated alone SH-SY5Y cells ( $p < 0.05$ ). Isomitraphylline demonstrated the decrease ROS levels to 301.78% and 238.92% for 10 and 20  $\mu\text{M}$  concentrations, respectively. Mitraphylline influenced the reduction of ROS levels by 295.09% at 10  $\mu\text{M}$  and 255.54% at 20  $\mu\text{M}$  ROS levels. The 1  $\mu\text{M}$  concentration did not show any significant ROS level reduction in comparison to the  $\text{H}_2\text{O}_2$ -treated alone SH-SY5Y cells ( $p < 0.05$ ). The intracellular ROS level of the alkaloids alone was also measured after 6 h of incubation (no oxidative stress). The ability of the alkaloids to reduce the ROS generation in  $\text{H}_2\text{O}_2$ -treated SH-SY5Y cells validated the neuroprotective activity, as shown in Fig. 5.

In Fig. 6, the mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ) was measured using 10  $\mu\text{M}$  and 20  $\mu\text{M}$ , as these concentrations could have protective effects against  $\text{A}\beta$  as indicated in Fig. 3. After alkaloids pre-treatment for 2 h, the cells were incubated with 5  $\mu\text{M}$   $\text{A}\beta$  for another 24 h. The  $\Delta\Psi\text{m}$  was measured using the tetramethylrhodamine, methyl ester (TMRE) stain. Treatment of the SY-SY5Y cells with the  $\text{A}\beta$  alone showed a significant decrease of  $\Delta\Psi\text{m}$  in comparison to the control cells ( $p < 0.05$ ). SH-SY5Y cells untreated with  $\text{A}\beta$  also gave comparable  $\Delta\Psi\text{m}$  values to the control cells. However, only the 20  $\mu\text{M}$  alkaloid +  $\text{A}\beta$ -treated cells presented significant increases in the MMP in comparison to the  $\text{A}\beta$ -treated alone SH-SY5Y cells. The  $\Delta\Psi\text{m}$  of the SH-SY5Y cells for the isomitraphylline gave 80.42% ( $\pm 3.66$ ), while mitraphylline demonstrated 77.34% ( $\pm 2.05$ ). In comparison, the  $\text{A}\beta$ -treated alone cells showed 64.79% ( $\pm 2.56$ )  $\Delta\Psi\text{m}$ . These results signify the potential attenuation of the mitochondrial dysfunction by the oxindole alkaloids by reducing ROS generation.

The structural diversity of small molecules has contributed to the researches in finding potential leads in drug discovery including neurodegenerative diseases. AD is a complex illness revolving in an interconnected genetic, biochemical, and metabolic pathways (Poloni et al. 2021; Reus et al. 2020). Hence, a molecule which targets more than one pathological hallmarks in AD is envisioned as a potential possibility in designing and developing effective drugs against AD. Several review papers have also reported on the ability of natural products with diverse structures as amyloid inhibitors (Rajasekhar et al. 2015; Tewari et al. 2018; Velandar et al. 2017). Majority of these natural products are flavonoids and polyphenols such as brazilin, curcumin, resveratrol, tanshinone, and epigallocatechin-3-gallate which also highlighted their anti-oxidant, anti-inflammatory, and metal chelating properties as possible mechanisms as potential multifunctional anti-amyloid compounds against AD (Rajasekhar et al. 2015; Velandar et al. 2017).

In this study, isomitraphylline and mitraphylline as potential candidates for further investigations in AD models are reported. Both alkaloids were able to demonstrate inhibition of the  $\text{A}\beta$  aggregation in the ThT assay. Their protective potential in the  $\text{A}\beta$ -induced or  $\text{H}_2\text{O}_2$ -induced neuroblastoma SY-SY5Y cells revealed inhibitions caused by the cytotoxic effects in the SH-SY5Y cells of either  $\text{A}\beta$  or  $\text{H}_2\text{O}_2$ . To validate these neuroprotective effects, both alkaloids showed reductions in the intracellular ROS level in  $\text{H}_2\text{O}_2$ -treated SH-SY5Y cells and mitochondrial dysfunctions in  $\text{A}\beta$ -induced SH-SY5Y cells. These parameters both associate to oxidative stress which is one of the pathological hallmarks associated with AD (Xia et al. 2019). Oxidative stress causes an increase in the level of reactive oxygen species leading to mitochondrial dysfunction. This

**Fig. 6** Effects of the alkaloids on the mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ). SH-SY5Y cells were pre-treated with the alkaloids for 2 h, and incubated with 5  $\mu\text{M}$   $\text{A}\beta_{1-42}$  ( $\text{A}\beta$ ) for 24 h.  $\Delta\Psi\text{m}$  was measured using the tetramethylrhodamine, methyl ester (TMRE) stain. Values are the mean  $\pm$  SD of triplicate experiments and expressed as % of the control. The (\*) indicates a significant difference compared to the  $\text{A}\beta$ -treated group alone at  $p < 0.05$



eventually leads to neuronal death-causing age-related illnesses including AD.

There is a limited study conducted on the neuroprotective effects of the oxindole alkaloids. This report is the first study on the neuroprotective effects of isomitraphylline and mitraphylline. Noteworthy, several related oxindole alkaloids including corynoxine, rhynchophylline, isorhynchophylline, and isoorynoxine, and the yohimbine indole alkaloids geissoschizine methyl ether, hirsuteine, and hirsutine have been investigated on their neuroprotective effects based on glutamate-induced cell death. The alkaloids rhynchophylline, isorhynchophylline, isoorynoxine, hirsuteine, and hirsutine showed protective effects on glutamate-induced neuronal death in cerebellar granule cells by inhibiting the  $\text{Ca}^{2+}$  influx (Shimada et al. 1999). The in vitro neuroprotective effects of these type of alkaloids project their potential as anti-AD drugs. However, in vivo studies are required to fully explore their therapeutic capacity against AD.

## Conclusion

Collectively, our findings indicated the neuroprotective effects of the oxindole alkaloids, isomitraphylline and mitraphylline, for their therapeutic potentials against AD. The inhibitions of amyloid- $\beta$  ( $\text{A}\beta$ ) aggregation by both alkaloids were demonstrated by the ThT assay, and their neuroprotective effects on  $\text{A}\beta$ -damaged neuroblastoma SH-SY5Y cells also supported their potentials. Reduction of oxidative stress was manifested from their protective effects on  $\text{H}_2\text{O}_2$ -induced SH-SY5Y cells. Both alkaloids reduced the ROS levels and mitochondrial dysfunctions. Hence, these alkaloids could be further investigated and developed as potential therapeutic agents for the treatment of AD.

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**Author contributions** Conceptualization, MAT and SSAA; methodology, MAT; formal analysis, MAT; writing—original draft preparation, MAT; writing—review and editing, MAT and SSAA; project administration, MAT and SSAA; funding acquisition, SSAA All authors have read and agreed to the published version of the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethics approval and consent to participate** The article does not contain any studies involving human participants or animals.

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