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Original Research Article

Preliminary investigation on the effect of methanol leaf extract of *Duranta repens* on enzymes of cholinergic and monoaminergic systems critical to neurodegeneration in rats *in vitro*

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Abstract

Purpose: *To evaluate the neurological effects of Duranta repens leaf extract by assessing its impact on monoamine oxidase-B (MAO-B) and acetylcholinesterase (AchE).*
Methods: *Rat brain homogenates were used as enzyme sources. The activity of MAO-B was determined spectrophotometrically using a kinetic method based on benzylamine as substrate, while the ability of the extract to inhibit AchE activity was assessed using enzyme-linked immunosorbent assay (ELISA).*
Results: *Compared to the standard drug (donepezil) the extract demonstrated high potency (IC_{50} = 9.30 mg/dL for the extract and 36.90 mg/dL for donepezil) in inhibiting AchE activity. Similarly, it exhibited a strong inhibitory effect against MAO-B (IC_{50} = 2.45 mg/dL for the extract and 11.50 mg/dL for donepezil).*
Conclusion: *The findings suggest that D. repens leaf extract has significant potential in reducing neuron loss. Further investigations to identify the specific phytoconstituents responsible for the inhibitory effects of the extract on MAO and AchE would be required.*

Keywords: *Neurodegenerative diseases, Duranta repens, Chikadoma plant, MAO-B, AchE*

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INTRODUCTION

Neurodegenerative diseases (ND) are disease states that are defined mainly by the loss of neurons. In the majority of brain-related illnesses, this is the primary pathophysiological alteration [1]. Major depressive disorder (MDD) is regarded as a neurodegenerative disease thought to be linked to neuronal atrophy in the hippocampus CA3 region and a decrease in neurogenesis within the hippocampus dentate gyrus [2]. Alzheimer's disease (AD), regarded as the most researched age-related condition, is primarily characterized by a progressive decline in cognitive functions, particularly memory [3]. White matter lesions are a hallmark of multiple sclerosis and it is regarded as a central nervous system (CNS) auto-inflammatory illness [4] while Parkinson's disease (PD) and Huntington's disease (HD) are instances of striatal motor

diseases. These are linked to severe motor dysfunction, with PD exhibiting tremors and slow/unsteady movement and HD exhibiting abnormal, uncontrolled movement.

There are now several recognized methods for treating neurodegenerative diseases that either address the etiology and course of the illness or reduce its symptoms. These include the use of donepezil, galantamine, rivastigmine and tacrin, the first choline esterase inhibitor approved for AD but later discontinued due to its hepatotoxicity [1]. In June 2021, Aducanumab, the first disease-modifying agent, was approved for AD as an antibody targeting amyloid-beta (Aβ) plaques [5]. For PD, management strategies either increase dopamine levels (levodopa + carbidopa combination, pergolide, selegiline) or decrease acetylcholine levels (benztropine, orphenadrine) [6].

All neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), sometimes referred to as Lou Gehrig's disease, motor neuron disease and Parkinson's disease (PD), have their dementia-related symptoms reduced by donepezil and galantamine [1]. Though there have been documented successes with well-known synthetic agents, their therapeutic acceptance is still limited due to a variety of concerns regarding their long-term benefits and the substantial economic harm they cause to society, particularly in low-, middle- and third-income countries like Nigeria. One of the main goals of neuro-ethno pharmacology is to find natural or herbal medicines that may be used as a viable alternative for reversing or stopping neurodegeneration [7].

Duranta repens possess some interesting prospects in this respect. It belongs to the Verbenaceae family and its English name is yellow bush [8]. In Nigeria, it is known as the Chikadoma plant [9] and it is commonly used as a decorative plant in homes, schools, hospitals and the hospitality sector for both aesthetic and therapeutic purposes. Due to the abundance of phytochemicals, *D. repens* is used to treat inflammation in Nigeria [9] and has been shown to have hypoglycemic [10], antiplasmodial [11] and enzyme inhibitory [12] properties. It has also been shown that *D. repens* contains three C-alkylated flavonoids along with (+)-3, 13-clerodadiene-16, 15-olid-18-oic acid and (+)-hardwickiic acid [10], two new flavonoid glycosides along with five known flavonoids [11] and a new triterpenoid with 14 known compounds that includes one flavonoid, four iridoides, one phenyl ethanoid glycoside, and eight of these compounds [13].

There is a definite link between oxidative stress and neurodegeneration. In previous studies, the effect of *D. repens* leaf extract on levels of some antioxidants and lipid peroxidation indices have been highlighted [8,14]. This study aimed to evaluate the inhibitory effects of *D. repens* leaf extract on neuronal loss by determining its activity against MAO-B and AchE enzymes.

EXPERIMENTAL

Preparation of plant extracts

The leaves of *D. repens* were collected from the vicinity of the University of Calabar, Nigeria, in August 2022. After being dried, the leaves were ground with an electric blender to powdery form and further subjected to extraction process [13,18]. The cold maceration technique was used to extract the physiologically active components from the leaves using 100 % methanol for 48 hours [19]. The methanol extract was filtered, then concentrated using a rotary evaporator at lower pressure and oven-dried.

Animals

A total of 20 animals, comprising of five rats each in four groups, (two- to three-month-old male Wistar rats) weighing 200 – 230 g were acquired from the Laboratory Animal House/Facility of the University of Calabar's Department of Pharmacology. The animals were acclimated to the precise work environment for two weeks and were kept with unrestricted access to standard pellets, water and other necessities in compliance with the International and Institutional Animal Care and Use Committee guidelines. In the experimental design, group 1 received MLE-DR, group 2 received Donepezil for AchE inhibition (percentage) determination group 3 received MLE-DR and Group 4 received Donepezil for MAO-B inhibition (percentage) determination.

Monoamine oxidase-B (MAO-B) activity assay

The capacity of *D. repens* leaf extract to raise or lower MAO-B activity (MA) was assessed using the kinetic approach, which was slightly modified from Akomolafe *et al* [15] and used a UV spectrophotometer set at 250 nm with benzylamine as the MAO-B substrate. The enzyme activity was determined using Eq 1.

$$MA = \Delta A*RV*1000/32.2*SV*0.5 \quad \ldots\ldots\ldots\ldots (1)$$

Table 1: Inhibition of AchE and MAO-B activities by different concentrations of MLE-DR compared to donepezil

Concentration (mg/mL)	AchE inhibition (%)		MAO-B inhibition (%)	
	MLE-DR	***Donepezil***	***MLE-DR***	***Donepezil***
0.5	7.4±0.03	2.4±0.08	96.6±0.10	77.4±0.05
1	10.9±0.08	3.5±0.04	96.9±0.02	88.3±0.02
2	22.9±0.07	5.8±0.10	97.3±0.09	88.7±0.06
4	33.6±0.04	9.5±0.02	97.7±0.10	88.9±0.02
6	38.1±0.09	11.1±0.06	98.6±0.10	89.6±0.05
8	43.6±0.03	12.8±0.01	99.6±0.05	89.0±0.04
10	49.5±0.03	14.6±0.02	99.8±0.02	89.9±0.08

where ΔA Is the difference in absorbance after 90 sec and 30 sec, RV is the total reaction volume and SV is the sample volume.

Acetylcholinesterase (AchE) activity assay

Enzyme-linked immunosorbent assay (ELISA) [16] was used to determine AchE inhibition. This approach primarily relies on the extract's capacity to block AchE, which hydrolyzes acetylcholine and disrupts brain signal transmission, resulting in a variety of neurodegenerative illnesses. In summary, 130 µL of PBS (0.1 M, pH 7.4) and 10 µL of rat brain homogenate were combined with 20 µL of the sample or standard (donepezil) at a different dose or blank (dd water) on an ELISA 96-well plate. For the sample blank,10 µL of PBS was used instead of the rat brain homogenate. After mixing the contents of the plate with a shaker and incubating it for 20 min at 37 ^{o}C, 5 µL of ATCL (75 mM in dd H_2O) was added to each well and incubated at 37 ^{o}C for 15 minutes. Finally, 10 µL of DTNB (Ellman's reagent; 0.32 M in phosphate buffer, pH 7.4) was added to the wells. Absorbance was monitored at 405 nm after 5 minutes with ELISA. The percentage of AchE inhibition (I %) was calculated using Eq 2.

I (%) = {(ABL–AT)/ABL}100 …… (2)

where A_{BL} and A_T are the absorbances of blank and sample/standard (donepezil), respectively.

Additionally, IC_{50} was calculated as the sample and the donepezil dose needed to impede AchE activity by 50 %.

Statistical analysis

Statistical analysis of the data was conducted using SPSS software (version 25). Analysis of variance (ANOVA) was used to ascertain the differences between groups. The data are shown as mean ± standard error of the mean (SEM), and a *p*-value less than 0.05 was deemed statistically significant.

RESULTS

The *D. repens* leaf extract exerted a strong inhibitory percentage against MAO-B and inhibited AchE activity (Table 1).

The IC_{50} values for AchE (9.30 mg/mL) and MAO-B (2.45 mg/mL) were notably lower than those of the reference drug, donepezil (36.90 and 11.50 mg/mL, respectively), highlighting its superior potency (Table 2).

Table 2: IC_{50} values of MLE-DR and donepezil that inhibited AchE and MAO-B activities

Medication	IC_{50}AchE for (mg/mL)	IC_{50} for MAO-B (mg/mL)
MLE-DR	9.30	2.45
Donepezil	36.90	11.50

DISCUSSION

The study's findings demonstrated that *D. repens* leaf extract had a strong inhibitory percentage against MAO-B and was highly effective in inhibiting AchE activity which is linked to the presence of phytoconstituents found in the plant [9-13]. Methanol leaf extract of *D. repens* (MLE-DR) and donepezil had inhibitory concentrations (IC_{50}) of 9.30 and 36.90 mg/dL, respectively against AchE and 2.45 and 11.50 mg/dL, respectively against MAO-B. However, no study has linked MAO-B and AchE to *D. repens* or its extract. Nevertheless, earlier research on other herbal extracts supported the repressive action of flavonoids and phenolics, which are substances that are abundant in *D. repens* [9-11].

The AchE-obstructing properties of phenolic substances, primarily flavonoids, at high concentrations have been identified [17]. There are several components in *D. repens* leaf extract that have a neurological function. The phenolic OH groups have the ability to scavenge reactive oxygen species (ROS), decrease inflammation in neurodegenerative illnesses and alter the levels of acetylcholine in the brain's cortex and

hippocampus. This study shows that leaf extracts with strong antioxidant activity might suppress AchE, which is consistent with the findings of Sekeroglu *et al* [18].

CONCLUSION

This study shows that *D. repens* leaf extract exhibits significant neuroprotective potential by inhibiting AchE and MAO-B activities. These findings support the traditional use of *D. repens* in managing neurodegenerative conditions. Further investigations to identify the specific phytoconstituents responsible for the inhibitory effects of the extract on MAO and AchE would be required.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Sylvester C Ohadoma: Conceptualization and supervision. Sylvester C Ohadoma, Ezechukwu I Nwokoma, Lapah P Takem, Ekaette S Udoh, Arit Umoren carried out the literature review, experimental work and manuscript drafting. All authors reviewed and approved the final manuscript. All liabilities with regard to claims relating to this article's content will be borne by the authors.

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