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A Comparative Free Radical Scavenging Evaluation of Amantadine and Rasagiline

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To evaluated and compare the intrinsic antioxidant ability of amantadine and rasagiline drugs using *in-vitro* diphenyl-1-picrylhydrazyl assay method. diphenyl-1-picrylhydrazyl assay method was used to compare the antioxidant activity of rasagiline and amantadine. At lower concentrations (200 - 400 μg/ml), there was a definite difference between amantadine and rasagiline with amantadine showing better antioxidant activity over rasagiline. But at higher doses (600 - 1000 μg/ml) both their antioxidant free radical scavenging activity were comparable. This study proved the intrinsic activity of rasagiline and amantadine which may be beneficial in attenuating the oxidative stress pathways, which were considered responsible for many degenerative diseases.

Keywords: Parkinson's disease, Reactive oxygen species, Amantidine, Rasagiline, 2,2-diphenyl-1-picrylhydrazyl assay.

Parkinson's disease is the neurodegenerative disorder affecting more than 1% of population aged above 65 years[1]. Most of the PD patients exhibit symptoms after 50-60% loss of dopaminergic neurons in substantia nigra pars compacta (SNpc)[2,3]. Free radicals or reactive oxygen species are certainly generated in living cells. They may also originate from external source. These free radicals were a known etiological factors for degenerative disorders[4].Antioxidants are the endogenous or exogenous compounds which fight free radical generation by intervening in pathways of oxidation[5]. Health and vigour of the biological system was known to be decided by the balance between antioxidants and oxidants[6]. The same was also postulated with "oxidative stress hypothesis". Drechsel & Patel (2008), Liddell *et al*., (2013) have stated that oxidation of dopamine produces ROS species and causing chronic oxidative stress in SNpc dopaminergic neurons[7,8]. Surendran, S., & Rajasankar, S. (2010);Ghanta, Mohankrishna, et al.,(2018) have shown oxidative stress in PD caused due to increasedglutamate toxicity, lipid peroxidation, protein oxidation and DNA damage in SNpc[9,10]. Treatment for PD included dopamine supplementation as gold standard with L-dopa and dopaminergic agonists since many years. Other drugs acting as enzyme inhibitors also aid in dopamimetic therapy[11-13]. Selegiline and Rasageline decrease dopamine degradation by inhibiting monoamine oxidaze-B (MAO-B) enzyme. Amantadine,

a tricyclic aminoadamantanes, synthetic drug acts as N-methyl D-aspartate receptor (NMDA) non competitive antagonist, antimuscarinic and proved beneficial in PD treatment[14-16]. Apart from the above said mechanisms of amantadine and rasagiline drugs, this study have aimed to evaluate and compare the intrinsic antioxidant activity of these two drugs through *in-vitro* anti oxidant assay.

MATERIALS AND METHODS

Drugs and Reagents

Amantadine hydrochloride (Amantrel Chennai, Cipla), rasagiline mesylate (Rasalect, Sun Pharma, Chennai), butylated hydroxytoluene (BHT) (Sigma Aldrich USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (DPPH sigma Aldrich USA) were purchased. The drugs were mashed and dissolved in dimethyl sulfoxide (DMSO) (DMSO Sigma Aldrich USA). Other reagents used in this study were of high grade available commercially.

DPPH Assay

This assay was performed with standard method followed by many studies[17-21]. Absolute methanol (3.7 ml) was allocated in all test tubes along with blank. 100µl of absolute methanol was added to blank tube. Then 100µl of BHT was added to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. Finally 200µl of DPPH reagent was added to all the test tubes at room temperature condition for minimum of 30 minutes then, checked absorbance of all samples 517nm. BHT was used as the standard drug in the study. The percentage (%) inhibition and EC_{50} values are calculated by using the formulae

$$\text{DPPH scavenged (\%)} = [\,(A_{\text{initial DPPH}} - A_{\text{final DPPH}}) / A_{\text{initial DPPH}}\,] \times 100$$

The EC_{50} value, which represents the concentration of drug that gives rise to a 50% reduction in DPPHabsorbance, was determined by linear regression analysis.

RESULTS

The % inhibition of amantadine was found to be 16.1%, 49.4%, 62.9%, 71.9%, and 89.9 % inhibition while that of rasagiline was found to be 11.9%, 25.6%, 61,6%, 68.7% and 85.6% inhibition compared with that of standard BHT which had 58.6%, 88.9%, 96.1%, 97.9% and 99.4% inhibition at concentrations of 200, 400, 600, 800, 1000 µg/ml respectively. This was depicted in Figure 1.

DISCUSSION

In order to evaluate the intrinsic antioxidant property of amantadine and rasagiline, DPPH assay was performed which is a standard technique,developed by Blois (1958)[22], for antioxidant *in-vitro* assay.The principal of this assay is based on reduction of DPPH, a constant free radical. The colour of DPPH solution is purple due to the presence of free/an odd electron. When DPPH and antioxidants react the stable free radical get reduced to DPPH-H in the company of a hydrogen donor .This results in yellow decolourization as the absorbance reduced from the DPPH radical to the DPPH-H form. This reduction is proportional to the quantity of electrons paired[22]. Both the drug compounds showed dose dependent increase in free radical scavenging activity. The activity was evident in lower doses (200 ug/ml) and showed a steady increase up to 1000ug/ml which was the highest dose tested. At lower concentrations, there was a definite difference between amantadine and rasagiline with amantadine showing better antioxidant activity over rasagiline. But at higher doses, the antioxidant free radical scavenging activity were comparable between the two drugs. This intrinsic antioxidant activity of these drugs could be characterized to the chemical structures of the drug compounds[23]. As oxidative stress caused due to free radicals was evidenced both clinically and preclinically in PD, this assay could reveal the drugs capable of augmenting or enhancing the treatment of PD apart from their known mechanism of actions[24]. Rasagiline a known drug for treatment of PD, inhibits MAO-B enzyme and increases dopamine availability in SNpc and striatum by reducing the dopamine degradation. It was also shown to reduce oxidative stress in rodent models[25-28]. Structural activity studies revealed that propargyl moiety of rasagiline was related to the MAO-B enzyme inhibition activity while some other studies have also revealed the MAO-B independent neuroprotective activity of rasagiline[29-32]. This present study have proved the intrinsic antioxidant activity of rasagiline.

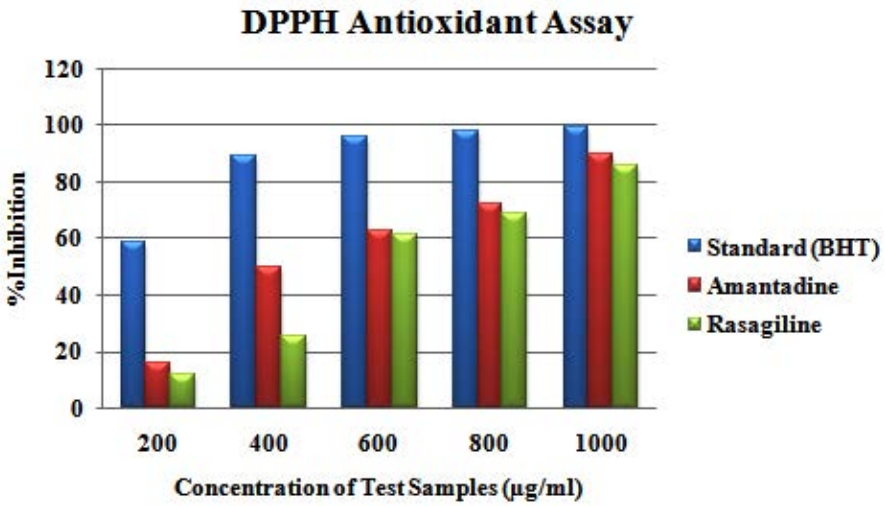

Fig. 1. DPPH antioxidant assay comparing antioxidant property between amantadine and rasagiline with control BHT

Amantadine is a known antiparkinsonian drug with NMDA receptor antagonist property[23-35]. Lupp et al. (1998) have reported the antioxidant property of amantadine in their *in-vitro* study[36]. In this present study also, amantadine have shown *in-vitro* antioxidant activity and proved to have free radical scavenging activity.

CONCLUSION

Both Amantadine and Rasagiline have shown *in-vitro* antioxidant activity in DPPH assays, which is one of the standard assays for antioxidant activity. Even though some works have previously stated into this path, there was a lack in the comparative research works among antiparkinsonian drugs along with an absence of healthy discussion on the theories that could clarify the antioxidant mechanisms of these drugs. The antioxidant action of amantadine and rasagiline may propose neuroprotection apart from their known mechanism of actions.

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