INTRODUCTION

Parkinson’s disease is a slowly progressing neurodegenerative disorder characterized by bradykinesia (slowing of movements), rigidity, increased muscle tone, tremors, and postural instability which is mainly due to loss or depletion of dopaminergic neurons in the substantia nigra [1]. The cause of this disease is multifactorial although the exact mechanism is still unknown. Recent studies have suggested that oxidative stress and abnormalities in the mitochondria can lead to increased free radical formation which might contribute to the pathogenesis of Parkinson’s disease [2]. Administration of an exogenous neurotoxin called MPTP is also said to cause this disease due to selective loss of dopaminergic neurons in substantia nigra [3]. The main aim of drug therapy in Parkinsonism is to provide symptomatic relief to the patient by use of drugs that can restore the dopaminergic and cholinergic balance that is disturbed in this case. Dopamine itself cannot be given as it cannot cross the blood-brain barrier (BBB), and its peripheral effects have no benefits in Parkinsonism patients. Hence, levodopa - the precursor of dopamine - is given, which can cross the BBB and get decarboxylated to dopamine on entering the brain. However, dopamine, which is formed from exogenously administered levodopa, is reported to be neurotoxic due to its conversion to some toxic products such as quinones and other free radicals [4,5]. These free radicals released might further increase the oxidative stress contributing to the pathogenesis of Parkinson’s disease. Moreover, patients on long-term levodopa therapy experience a lot of adverse effects such as “on-off” phenomenon, dyskinesias, wearing off phenomenon, hallucinations, delusions, sleep disturbances, and schizophrenia-like symptoms [6]. These shortcomings of levodopa lead to the need for newer therapeutic agents with a neuroprotective role in the treatment of Parkinson’s disease [7]. Now, dopamine agonists such as bromocriptine, ropinirole, pergolide, pramipexole, cabergoline, and rotigotine have said to have a neuroprotective effect in experiments conducted on animal models [8]. They have also reported to prevent the clinical progression of disease. Studies have shown that ergot derivatives of dopamine agonists such as bromocriptine and cabergoline have free radical scavenging property and possess antioxidant activity in vivo and in vitro. However, the main disadvantage of the ergot derivatives is increase in adverse effects such as anorexia, nausea, vomiting, vertigo, postural hypotension, painless peripheral vasoospasm (pasm of digits), peripheral edema, pleural fibrosis, and erythromelegia [9]. These ergot derivatives also have poor tolerability profile. These drawbacks of ergot derivatives are overcome by non-ergot derivatives such as ropinirole and pramipexole which have fewer side effects and better tolerability profile [10]. However, unlike the ergot dopamine agonists, very few studies have reported the neuroprotective role of non-ergot derivatives of dopamine agonists such as ropinirole, pramipexole, and talipexole. The neuroprotective role of these non-ergot dopamine agonists is also probably because of its free radical scavenging and antioxidant activity. Hence, this study was undertaken to evaluate the antioxidant and free radical scavenging activity of non-ergot dopamine agonists - ropinirole and pramipexole, novel agents used in the treatment of Parkinson’s disease using the in vitro 1, 1-diphenyl-2-picyrylhydrazyl (DPPH) assay.

METHODS

Chemicals

DPPH and drugs pramipexole and ropinirole were obtained from the Sigma Chemical Co. CRL, Bangalore. Ascorbic acid was obtained from the SD Fine-Chem. Limited, Biofarm, India.

DPPH free radical scavenging activity

DPPH radical scavenging activity was done using the method of Brand-Williams et al. [1995] [11-13]. The DPPH assay is based on the reduction of DPPH a stable free radical. The DPPH solution was prepared (0.004% w/v) in 95% ethanol. The reaction mixture containing 1 ml of DPPH solution (1 mM in ethanol) with 3 ml of different concentrations of ropinirole (50, 40, 30, 20, 10, and 5 µg/ml) in ethanol was shaken and incubated in dark for 20 minutes at room temperature. Ropinirole was soluble in ethanol, and its various concentrations were prepared by dilution method. The resultant absorbance was recorded at
517 nm using a spectrophotometer (HACH 4000 DU ultraviolet-visible spectrophotometer). This procedure was similarly repeated for different concentrations of pramipexole (50, 40, 30, 20, 10, and 5 µg/ml), and the resultant absorbance was recorded at 517 nm. Ascorbic acid was used as the reference standard and dissolved in distilled water to make the stock solution with same concentration (100 µg/ml). Control sample was prepared containing the same volume. 95% ethanol served as blank. The percentage of DPPH free radical scavenging was calculated using the formula:

\[
\text{Scavenging} = \frac{A_c - A_s}{A_c} \times 100
\]

Where, \(A_c\) is absorbance of control and \(A_s\) or \(A_t\) is the absorbance of standard or test. The inhibitory concentration (IC\(_{50}\)) value was calculated using Microsoft excels 2016. A scatter graph was made in excel, and the slope equation \(Y = aX + b\) was obtained. With this equation, the IC\(_{50}\) was calculated using the formula IC\(_{50}\) = (0.5 − b)/a.

RESULTS

At concentrations of 5, 10, 20, 30, 40, and 50 µg/ml the percentage of inhibition of DPPH free radical activity observed with ropinirole was 0.2256, 0.1486, 0.0869, 0.0745, 0.0629, and 0.0527, respectively. At the same concentrations, the absorbance observed with pramipexole was 0.2216, 0.1239, 0.0745, 0.0648, 0.0365, and 0.0294, respectively. Ascorbic acid which was the reference standard showed the absorbance of 0.1916, 0.1182, 0.0416, 0.0391, 0.0335, and 0.0261.

At concentrations of 5, 10, 20, 30, 40, and 50 µg/ml the percentage of inhibition of DPPH free radical activity observed with ropinirole was 7.66%, 39.22%, 64.46%, 69.54%, 74.24%, and 78.44%, respectively (Table 1). At the same concentrations, the percentage of inhibition observed with pramipexole was 9.33%, 49.32%, 69.55%, 73.49%, 85.07%, and 88.01%, respectively (Table 1). Ascorbic acid which was the reference standard had the percentage of inhibition of 21.61%, 51.66%, 83.01%, 84.02%, 86.32%, and 89.34% (Table 1). The IC\(_{50}\) value calculated was 16.29, 18.19, and 10.16 µg/ml for ropinirole, pramipexole, and ascorbic acid, respectively (Fig. 1-3).

DISCUSSION

Studies conducted earlier by Hauser et al., Gaki and Papavassiliou, and Niranjan [2,14,15] have shown that increased oxidative stress and free radical formation contributes to the pathogenesis of Parkinson’s disease. Hence, drug molecules which have free radical scavenging property will have the neuroprotective effect in patients with Parkinson’s disease. From our study, the free radical scavenging property as measured by DPPH method showed that ropinirole and pramipexole have got an effective free radical scavenging activity when compared with ascorbic acid which was used as a standard (Fig 4). The results of this study are analogous with the studies conducted earlier by Joyce et al., Park et al., and Tanaka et al. [16-18]. Hence, from our study and other studies conducted earlier, it is evident that even non-ergot derivatives such as pramipexole and ropinirole might have additional beneficial effects in the disease because of its antioxidant activity apart from acting as selective D2/D3 dopamine receptor agonists.

CONCLUSION

In this study both ropinirole and pramipexole showed increasing free radical scavenging activity with increasing concentrations. Among the two drugs, pramipexole and ropinirole which were subjected to in-vitro DPPH
Fig. 4: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of ropinirole, pramipexole, and ascorbic acid

assay, pramipexole showed the slightly higher percentage of inhibition when compared to ropinirole at all concentrations. However, the IC_{50} levels of both the drugs were almost similar. Hence, this study shows that pramipexole and ropinirole have got good free radical scavenging activity and is non-inferior to each other and could play a role of neoadjuvant antioxidant in a wide variety of neurodegenerative disorders.

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