Section-III

Chapter-5

DISCUSSION

5. DISCUSSION

The dopamine D_1 receptor is confined in high concentrations in a few anatomically different parts of the rodent, monkey and human basal ganglia [81, 82]. A far reaching assemblage of behavioral and biochemical confirmation show a contribution of the dopamine D_1 receptor in the functioning of the rat basal ganglia. In any case, the role(s) for the dopamine D_1 receptor in the functioning of the monkey and human basal ganglia stays dark [83-85]. In this manner, **SKF 38393**, the most broadly utilized D_1 agonist, is without remedial effect for the Parkinsonian manifestations of either MPTP-treated primates or people with idiopathic Parkinsonism [86-87]. As of late, the synthesis of a chain of isochroman derivatives that are potent, specific and long-acting agonists for the dopamine D_1 receptor has been accounted for [88-89]. **A-77636** ((1*R*, 3*S*) 3-(l'-adamantyl)-1-aminomethyl-3,4-dihydro-5,6-dihydroxy-l*H*-2-benzopyran hydrochloride) is an alternate dopamine D_1 receptor-specific isochroman that is an agonist both *in vitro* and *in vivo*. Moreover, previous studies demonstrated that **A-77636** eases the Parkinsonian manifestations of marmosets treated with the neurotoxin MPTP. These observations and the other observations published by Taylor et al. [90] display that the dopamine D_1 receptor plays an important part in the working of the extrapyramidal nervous system of primates.

The participation of the dopamine D_l receptor in the performance of the extrapyramidal nervous system is in agreement with many observations; high concentrations of D_1 dopamine receptors arise in several sites inside the basal ganglia, most notably the substantia nigra pars reticulata and caudate putamen [91-93]; the construction of a second messenger (cyclic AMP) was stimulated by D_1 agonists in the tissue from either the substantia nigra or the caudate-putamen [94].

Similarly, emulating damage of the striato-nigral dopaminergic neurons with 6-OHDA, D_1 receptor (not D_2 receptor) was stimulated causing utilization of glucose in the substantia nigra pars reticulata and the entopeduncular nucleus [95-97]. *L*-DOPA, the most broadly utilized medicine for PD, likewise builds glucose use in this experimental model [98] awhile ago, an alternate D_1 agonist isochroman, **A68930**, was demonstrated to additionally increment nigral growth of 2-deoxyglucose [89]. These perceptions point to a part for dopamine D_1 receptors in the physiology of the extrapyramidal sensory system. Thus, the dopamine D_1 receptor turns into a satisfactory focus in the rational design of anti-Parkinsonian agents.

In aggrement with the above reports, a series of benzazepines were synthesized at the Pharmaceutical Chemistry laboratory. They were assessed for D_1 agonistic potential using

isolated mesenteric artery preparation. Compound (**3d**) came out as a potent D_1 agonist. It was further assessed against 6-OHDA induced injury in human SH-SY5Y neuroblastoma cell lines. The compound (**3d**) showed neuroprotection in the cell culture.

According to previous reports, DNS treated 6-OHDA induced unilateral lesioned rats in the medial forebrain bundle showed vigorous contralateral turning. The DNS induced rotational behavior emerges to be the case of *in vivo* D_1 receptor stimulation. The rotation induced by DNS was fully blocked by D_1-selective antagonist **SCH-23390** while no effect on rotation was shown by D_2-selective antagonist raclopride [99]. The compound (**3d**) which was proven D_1 agonist in preliminary *in vitro* studies was further evaluated using 6-OHDA unilaterally lesioned Parkinson's rat model. Compound (**3d**) significantly increased number of contralateral rotations in 6-OHDA unilateral lesioned rats. This showed that the compound (**3d**) is having affinity towards D_1 receptor and the rotations were due to D_1 receptor stimulation.

6-Hydroxydopamine (6-OHDA) is a neurotoxin that gives functional animal models of PD by inducing the dopamine (DA) neurons' degeneration in the substantia nigra (SN) pars compacta (SNpc) and injured their nerve endings in the striatum [100-101]. Similarly, 6-OHDA decreased DA level in rat striatum. The DA level was significantly increased by treatment of the compound (**3d**) in 6-OHDA induced Parkinson's rat brain. These results further pointed out towards the D_1 agonistic potential of the compound (**3d**).

Apoptosis is likewise a pigeonholed biological procedure through which cells are disposed off throughout cell development and because of injury. Under numerous neurodegenerative diseases, apoptosis has been found to altogether help neuronal cell death [102]. PD may be likewise categorized by progressive DArgic neurons degeneration by apoptosis of the nigrostriatal organization. Furthermore, mitochondrial or Fas- pathways mediate caspase-3 activation induced by 6-OHDA in cells [103]. Even though various mechanisms have been proposed in the progression of PD, a thought has been made that oxidative stress plays a part in the progression of the neurodegenerative development in PD [104, 105].

Capable detoxification pathways have been recognized in various cells to control the hazardous effects of oxidative stress. Of the different antioxidants in the brain, the GSH framework is especially critical in controlling cell redox states and is the essential protection pathway for peroxide expulsion from the brain [106, 107]. In addition, the MDA levels may be dominatingly reliant on ROS levels, including hydroxyl radicals [108]. Hence, MDA (a non radical product), which is cytotoxic in itself from LPO, may play an essential role in

the altered progression induced by i.c.v. unilateral injection of 6-OHDA. Also, apoptosis seems, by all accounts, to be anticipated by expanding the intracellular GSH levels in quite a few models of oxidative stress-induced apoptosis [109]. After getting significant results in behavioural studies, the compound (**3d**) was further evaluated for its antioxidant parameters. The compound (**3d**) significantly modified the oxidative parameters viz. GSH, MDA, SOD and catalase. Treatment with the compound (**3d**) significantly increased GSH, SOD and catalase levels in 6-OHDA induced Parkinson's rat brain. Additionally, treatment with the compound (**3d**) significantly decreased MDA level in 6-OHDA induced Parkinson's rat brain. The results pointed out that the compound (**3d**) is having antioxidant property.

One of the most important apoptotic activators is the caspase family. As a rule, caspase-3 is measured to play an essential part in the final regular pathway of apoptosis. Fas (one of the death receptors present on surface of the cell) corresponds to a pathway practically solely controlled by caspase. According to this pathway, binding of ligand to the death receptor leads to accumulation of a sequence of proteins, which leads to activation of pro-caspase-8 [110, 111]. The following proceedings are the strongest indication that caspases work in cascades, with caspase-8 activating caspase-3, which further activates former caspases and cleave different substrates. Caspase-3 sets free caspase-dependent endonuclease (one of the substrates) from its inhibitor in the cytoplasm which then consequently penetrates in the nucleus, where it slices DNA into oligonucleosomal fragments [112]. In an alternate pathway of apoptosis, mitochondrial dysfunction arises during apoptosis which leads to the release of cytochrome c from the mitochondria into cytosol, where it attaches to apoptotic protease activating factor 1(Apaf-1) that restrains binding locates for cytochrome c [113, 114]. The complex then initiates the apoptosome, selects and ties pro-caspase-9 by Apaf-1 [115, 116]. Further, caspase-3 has been activated by mature caspase-9 released from the multimeric complex. Therefore, 6-OHDA provokes caspase-3 activation mediated by an after effect of either the mitochondrial- or Fas-pathways [103, 109]. In agreement with the above reports, it was planned to evaluate whether the compound (**3d**) has effect on caspase level. For this, the animals were sacrificed and the brain sections were utilized for immunohistochemical analysis for cleaved caspase-3. Confocal microscopic images demonstrated that 6-OHDA significantly increased cleaved caspase-3 positive cells in substantia nigra region. Compound (**3d**) significantly decreased cleaved caspase-3 positive cells in substantia nigra region of 6-OHDA induced Parkinson's rat brain. This authenticated that the compound (**3d**) is D_1 agonist and also showed neuroprotection through antiapoptotic

mechanism. In conclusion, as there are very few dopamine D_1 agonist medications available to treat PD, compound (**3d**) could be a potential drug candidate to treat PD.