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ORIGINAL ARTICLE

Neuroprotective effect of diclofenac on chlorpromazine induced catalepsy in rats

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Abstract

Neuroinflammation plays a key role in progressive degeneration of dopaminergic cells. Upregulation of prostaglandins and free radicals formation are involved in the mechanisms of cell death in Parkinson's disease (PD). The present study aimed to investigate the neuroprotective effect of diclofenac against chlorpromazine (CPZ) induced catalepsy and motor impairment in mice. Adult Wistar rats treated with CPZ (3 mg/kg/day, IP) were orally dosed with diclofenac and L-dopa/carbidopa for 21 days. Catalepsy was measured after 21 days of dosing by using standard bar test at 30, 60, 90, 120 and 180 min then motor performances were assessed via open field test and wire hanging test. Histopathological investigation and determination of dopamine (DA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) levels of rat's brain was also carried out. We found that CPZ treated group exhibited reduced motor impairment after 21 days of treatment in open field and wire hanging test ($P < 0.01$) as compared to control group. The cataleptic scores of CPZ treated rats were also significantly increased ($P < 0.01$) after 21 days of chronic dosing, however diclofenac treated groups showed significant reduction in cataleptic scores with improved motor performances. Histopathology of CPZ treated rats showed marked degeneration with architecture distortion in the mid brain region. Dopaminergic degeneration is confirmed by neurochemical results that showed reduced amount of dopamine and DOPAC levels in mid brain. Moreover, histopathological slides of diclofenac treated rats showed improved architecture with reduced gliosis of mid brain region as well as improved dopamine and DOPAC levels were achieved after 21 days dosing of diclofenac. Taken together, the present work provide an evidence that diclofenac ameliorated behavioral performances by mediating neuroprotection against CPZ induced PD via preventing dopaminergic neuronal cell death.

Keywords Parkinsonism · Chlorpromazine · Diclofenac · Neuroprotection · Dopamine and DOPAC (3,4-Dihydroxyphenylacetic acid)

Introduction

Parkinson's is common disease among elderly peoples. In USA about 10% elderly population i.e. 60 years of age and above usually suffers with any neurodegenerative diseases especially Parkinson's disease (Homykiewicz 1998). Parkinson's disease is characterized by tremors, rigidity, bradykinesia, postural instability and abnormal gait (Marsden and Calne 1994). It is associated with dopaminergic neuronal cell damage in basal ganglia especially in substantia nigra (SN) that control body movement and muscular coordination (Fahn and Przedborski 2000). Recent studies show that neuroinflammation is a major hallmark of Parkinson's disease (PD) (Mosley et al. 2012). According to recent researches, the release of proinflammatory mediators from neuronal cells i.e. microglia is now been recognized as a key factor for Parkinson's disease (Chen et al. 2010). Microglia are densely present in striatum and substantia nigra, where they show over expression of prostaglandins (PGs), tissue necrotic factor (TNF)-α and interleukin (IL) during neuro inflammatory processes. Inflammatory mediators responsible to initiate oxidative stress within the brain by generating reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS) species (Tansey and Goldberg 2009; Krause and Müller 2010; Ng et al. 2008). Whitton in 2007 demonstrated over expression

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of cyclooxygenase (COX) enzymes and its derivatives especially $PGE_{2,}$ in mid brain linked to PD. Raised levels of PGE_2 and other cytokines in mid brain region regulate the Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme system which further generates ROS and free radicals that in turn produce oxidative stress (Hernandes and Britto 2012). This results in neuronal degradation and dopaminergic cell death (Ransohoff and Perry 2009; Zhang et al. 2005).

Various cataleptic animal models are used for better understanding of the Parkinson's disease and development of its appropriate therapy. In order to evaluate neuroprotective effect of diclofenac, in the present study CPZ was used to induce Parkinson's like symptoms in animal model. Chlorpromazine is a classic antipsychotic drug known for its propensity to cause extrapyramidal symptoms, impaired memory and catatonia owing to blockade of striatal dopamine D2 receptors (Riaz et al. 2018; Naeem et al. 2017; Khatoon et al. 2016; Nair et al. 2007). It produces oxidative stress in brain which results in producing motor defects (Sandhu and Rana 2013; Terry et al. 2008). In addition to dopamine D2 receptors blockage CPZ also produces a state of catalepsy in human or animals by reducing dopaminergic transmission in basal ganglion (Cairo et al. 2006; Dauer and Przedborski 2003). Therefore, those agents that reduce neuroinflammation, oxidative stress and improve dopaminergic transmission could be beneficial.

Inflammation is the hallmark of Parkinson's diseases that leads to the nigrostriatal dopamine system degradation. Although dopamine replacement therapy can alleviate symptoms of the disorder, however there is no proven therapy to halt the underlying progressive degeneration of dopamine-containing neurons. Recently, increasing evidence from human and animal studies has suggested that neuroinflammation is an important contributor to the neuronal loss in PD. We have designed current study to investigate whether pharmacological inhibition of neuronal inflammation will be able to safely reverse or slow the course of Parkinson's disease. NSAID's can be the better option to reduced neuronal inflammation, as several studies suggests possible therapeutic role of NSAIDs in the treatment of neurodegenerative diseases (Wang et al. 2015; Pizza et al. 2011; Glass et al. 2010). The available treatments like levodopa, carbidopa and other antiparkinson's drugs effectively overturns only the sign and symptoms of PD. They improves the level of dopamine but does not interfere with neuroinflammation (Khatoon et al. 2016; Karch 2009). Diclofenac is a widely used non-steroidal anti-inflammatory drug that rapidly cross blood brain barriers, inhibit neuronal cyclooxygenase enzymes (Bendlin et al. 2010; Parepally et al. 2006) and act as an antioxidant. It also activates nuclear factor peroxisome proliferator-activated receptor (PPARγ) which is neuroprotective in the case of Parkinson's disease (Episcopo et al. 2010). Diclofenac was developed in 1973 as an analgesic agent, and since then it has been commonly prescribed worldwide. The effect of diclofenac was studied for its neuroprotective and antidepressant effect in different invivo and invitro models (Makunts et al. 2018; Milusheva et al. 2008). It was discovered that in rat models, diclofenac restored interferon (INF)-alpha induced increase in monoamine neurotransmitter turnover and restored dopamine levels (Jaturapatporn et al. 2012; De La Garza and Asnis 2003). Diclofenac prevents neuronal loss and cellular damage in Parkinson's model through its anti-inflammatory effects (Terzi et al. 2018). Further, Diclofenac promotes PPARγ production and protect neurons from inflammation and N-methyl-D-aspartate (NMDA) induced cytotoxicity (Kaplan et al. 2017; Landreth et al. 2008; Walker and Lue 2007; Combs et al. 2000). Carta and Pisanu (2013) explained PPARγ decreased the expression of neurotoxic factor in neurons thus preventing neuronal damage and limiting neurodegeneration.

Current study was designed to explore the role of diclofenac in providing neuroprotection and subsequent improvement in motor deficit in CPZ induced animal model of Parkinson's disease.

Material and method

Chlorpromazine (Sigma-Aldrich, MO, USA). L-dopa/Carbidopa (OBS-Pharma Company, Pakistan) and Diclofenac sodium (Novartis Pharma (Pak) LTD) both drugs were procured from local pharmacy and freshly prepared daily by dissolving in 0.9% normal saline and given orally. All chemicals of analytical grade were used in this experiment.

Experimental animals

In the present experiment two groups of forty adult Wistar rats were used to induce Parkinsonism by chlorpromazine. The age of selected animals (rats) was 6 to 8 weeks. Selected animals weighs ranged from 150 to 250 g with equal sex distribution. Animals were kept under standard environmental conditions, temperature: 21 ± 1 °C, humidity: 55 ± 5% with 12:12 h light/dark cycle at the Department of Pharmacology, Faculty of Pharmacy, University of Karachi. The animals were maintained with free access to water and regular rat diet ad libitum. They were caged in pair and were acclimatize at least 7 days before the start of experiment. Animals were handled as per specification provided in Helsinki Resolution 1964. The plan followed for animal experiments was approved by BASR University of Karachi under resolution No. 02(50).

Experimental protocol

Adult Wistar rats were randomly selected for this study and divided into 2 groups, group I and group II respectively. Group I served for the assessment of catalepsy while group II for open field and wire hanging test. Animals were randomly selected for histopathological and neurochemical assessment from both groups. Each group was further divided into 4 subgroups termed as group A, B, C and D. Group A was set as control and received saline water via oral route. Group B received chlorpromazine (3 mg/kg/day IP) only and served as the negative control (Bigoniya and Rana 2005; Bishnoi et al. 2006). Group C received chlorpromazine (3 mg/kg/day IP) and was treated with oral suspension of diclofenac sodium (20 mg/kg per day) (De La Garza and Asnis 2003). Group D standard group received chlorpromazine (3 mg/kg/day IP) and was treated with suspension of L-dopa/carbidopa (30 mg/kg oral suspension). CPZ was administered 30 min before the administration of normal saline, diclofenac and L-dopa/carbidopa (Sandhu and Rana 2013) to all treated groups.

Behavioral studies were carried out at day 10 and day 21 at ambient temperature without any disturbance. At day 21, the brain of selected animals were collected by decapitation for further histopathological and neurochemical studies.

Motor evaluations

Open field test

To explore spontaneous locomotor activity of all treated animals, open field test was selected. The apparatus was a square open field arena consisted of plastic board. The dimensions of the floor was 76 cm × 76 cm × 40 cm) with 25 equally spaced squares. Escaping of animals was prevented by surrounding walls which were painted in black. All the animals were individually placed in the central square for the duration of 10 min. All control and treated group animals were placed in the central square of the open Field separately for free exploration. Number of squares crossed were noted during 10 min time duration after the administration of control and test drugs at day 10 and day 21 (Sestakova et al. 2013).

Wire hanging test

Wire hanging test was used to evaluate grip strength of animals after CPZ induced brain injury. For this purpose, a 2 mm wire was stretched between two stainless steel posts of 60 cm height and the animals were trained to grab onto the wire as long as they can. All the control and treated animals were individually placed on stainless steel bar and allowed to grasp the wire. At day 10 and 21, time to drop off from the wire (latency to fall) of each animal was noted in seconds (Bonetto et al. 2015; Takahashi et al. 2009).

Catalepsy test

The cataleptic behavior of animals were evaluated by standard bar test method. Catalepsy, defined as a reduced ability to initiate movement and a failure to correct abnormal posture, was measured by means of the bar test. For this purpose, rats were positioned so that their hindquarters were on the bench and their forelimbs rested on horizontal wooden bar, 9 cm above from the bench. This procedure was conducted 30 min after drug administration. The length of time that rat maintained cataleptic position was recorded by stopwatch to a maximum of 720 s and carried out after 30 min, 60 min, 120 min and 180 min time intervals after administration of chlorpromazine and test drugs (Pires et al. 2005). The animals was considered to be cataleptic if the animal maintained the imposed posture for at least 20 s or more. The animals were tested on 21st day of the drug administration and only the greater duration of the immobility was considered significant.

Histopathological examination

At day 21st, after the completion of motor and behavioral tests all rats ($n = 10$ per group) were anesthetized using ether and their brain was removed quickly from the skull and washed with normal saline (ice cool). Brains were then stored in paraformaldehyde (10%) solution. Fresh tissue samples from the mid brain region of rat's brain were separated out. Each brain was serially cut into 30 μm thick sections and prepared for staining with hematoxylin and eosin (H and E). After staining, the sections were observed under bright-field microscope.

Dopamine and 3,4-Dihydroxyphenylacetic acid (DOPAC) estimation

At the end of the behavioral experiments, to estimate the striatal DA and DOPAC (DA metabolite) levels, animals from the different experimental groups were sacrificed. Mid brain was dissected out, weighed and were immediately dipped in chilled 0.9% *w*/*v* saline and stored at −76 °C or very low temperature. For estimation of DA and DOPAC mid brain sections were homogenized using perchloric acid (0.17 M) in a ratio of 10% w/v for 30 s using electric homogenizer to extract the DA and DOPAC from the tissue. The homogenate was kept aside for 15 min for whole protein precipitation and then transferred into the eppendorf tubes for centrifugation. Centrifugation was done at 1000 rpm for 5 min. Supernatants were filtered in another Eppendorf tube and centrifugation was repeated until clear solution was obtained. Filtrate was stored at low temperature of −76 °C till analysis.

HPLC assay

Electrochemical detection of concentrations of dopamine (DA) and dioxyphenylacetic acid (DOPAC) were obtained at 0.8 V operating potential using Shimadzu L-EC 6A detector of high performance liquid chromatography with Electrochemical Detection (HPLC–EC) technique after the long-term administration of diclofenac and other treated drugs as well as of control animals (Takween et al. 1998).

Data analysis

The collected data was analyzed by using SPSS version 21.0. Values are expressed as Mean ± SEM and the comparisons between means of control and treated groups were carried out using one-way analysis of variance (ANOVA) followed by post hoc analysis by Bonferroni test. P value <0.05 was considered significant between all groups.

Results

Effect on open field activity

Chlorpromazine treated negative control group significantly deteriorate the motor performances in the open field test, as all rats of this group traveled less distance, showed immobility and crossed fewer no of squares at day 10 (29.10 ± 1.929, $P < 0.01$) and day 21 (6.61 ± 1.192, $P < 0.001$) of dosing in comparison to control and treated groups. Figure 1 shows that diclofenac significantly inverted CPZ induced motor impairment in rats as animal crossed increase no. of squares after 10 days (24.50 ± 1.79, P < 0.01) and 21 days (38.40 ± 1.95, P < 0.01) of dosing similar to standard drug L-dopa/carbidopa (23.30 ± 2.144, P < 0.01), (41.70 ± 4.14, P < 0.01).

Effect on wire hanging test

Diclofenac and L-dopa/carbidopa treated rats showed increased latency of hanging at 10th day of dosing (72.20 ± 3.81, $P < 0.05$), (76.90 ± 3.84, P < 0.05) respectively as compared to chlorpromazine treated group (64.50 ± 4.01, P < 0.05). At 21st day diclofenac treated and standard group showed highly significant wire griping strength and latency time (101.5 ± 3.59, $P < 0.001$), (108.2 ± 2.96, P < 0.001) as compared to chlorpromazine treated group (6.10 ± 0.621, P < 0.001). chlorpromazine treated rats showed significant (P < 0.001) reduction in hanging time and weak muscle strength after 21 days (6.10 ± 0.54) of dosing as compared with control and diclofenac treated group. Slight changes were also seen in latency to fall after 10 days of CPZ dosing but results were insignificants (Fig. 2).

Effect on catalepsy

Cataleptic behavior of all animals were evaluated at 21 days of dosing at 5, 60, 120 and 180 min interval. Post-hoc analysis by Bonferroni's test showed that chlorpromazine produced significant catatonia (inability to change posture) in negative control group after 5 min of dosing (11.6 ± 3.19) which then significantly increased by the time. The cataleptic scores increased to 109 ± 8.15 ($P < 0.001$) on 60 min, to 321.5 ± 26.47 (P < 0.001) on 120 min, and finally to 183.9 ± 17.93 ($P < 0.05$) on 180 min. While diclofenac treated group showed significant reduction in cataleptic score as indicated by the highly significant ($p < 0.001$) reduction in time spent on the bar in the bar test after 5 min (9.5 ± 2.03), 60 min (57.2 ±

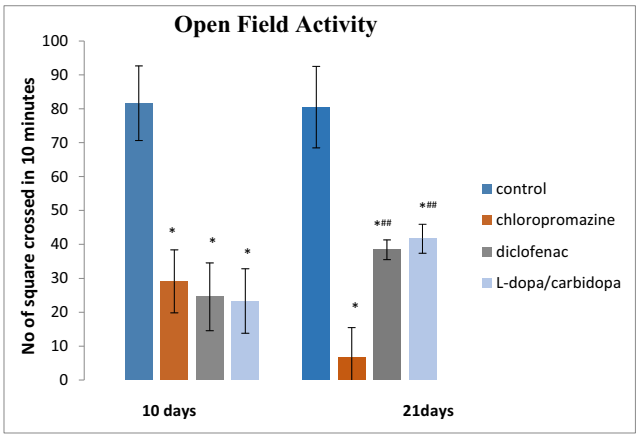

Fig. 1 Effect of diclofenac on Open Field activity. The effect of NSAIDs and chlorpromazine on Open field activity as compared to control group after 10 and 21 days. Values are $\overline{x}$ + SEM. ($n = 10$), df (1, 36). *$p < 0.05$, **$p < 0.01$, as compared to control group. #$p < 0.05$, ##$p < 0.01$, as compared to Chlorpromazine group

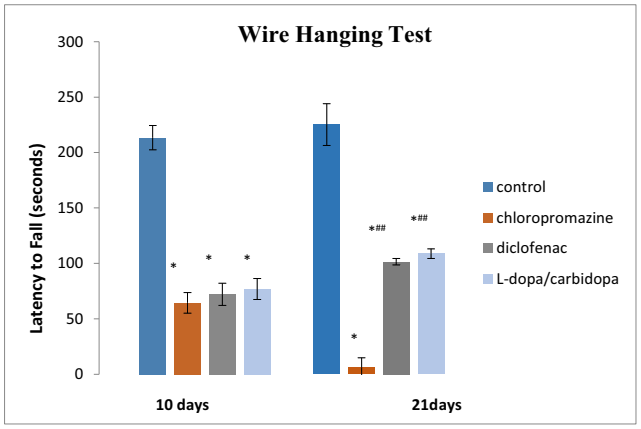

Fig. 2 Effect of diclofenac on Wire hanging test. The effect of NSAIDs and chlorpromazine on Wire hanging test as compared to control group after 10 and 21 days. Values are $\overline{x}$ + SEM. (n = 10), df (1, 36). *$p < 0.05$, **$p < 0.01$, as compared to control group. # $p < 0.05$, ##p < 0.01, as compared to Chlorpromazine group

9.36), 120 min (44.8 ± 5.37) and 180 min (14.7 ± 2.62) when compared with negative control (CPZ treated group) (Table 1).

These results showed cataleptic latency time was reduced by diclofenac treated group at day 21 and it was significantly decreased ($P < 0.001$) at different time interval in comparison to CPZ treated group.

Histopathological examination

Our histopathological findings (Fig. 3b) indicate that chlorpromazine produced neuronal degeneration in rat brains as CPZ treated groups showed marked neuronal degeneration. Neurons appeared in less numbers with indefinite boundaries, edema and gliosis with moderate darkly stained cells. These cells are indicative of oxidative stress when compared to control group. Histopathological pictures of diclofenac treated animals showed reduce edema, gliosis and presence of reduced neutrophils and few number of astrocytes that represents improved degeneration in diclofenac treated group. Figure 3d showed significant recovery of neuronal damage that confirms the neuroprotective effect of diclofenac.

Effect on dopamine and DOPAC levels

In order to assess the restoration of dopaminergic neurons' functional viability, DA and dihydroxyphenylacetic acid (DOPAC) levels were assessed in the mid brain region of the rat brains from all experimental groups. As shown in Table 2, CPZ treated group significantly reduced dopamine levels (176.87 ± 29.20) and DOPAC levels (232.96 ± 21.49) in comparison to control, diclofenac and standard group ($P < 0.01$). However dopamine and DOPAC levels were significantly higher ($P < 0.05$) in diclofenac treated group as compared to CPZ treated group at 21 days of treatment (207.56 ± 11.87), (356.48 ± 14.07) respectively. These results show that CPZ administration significantly reduces DA and DOPAC levels in mid brain region of rat brains and treatment with diclofenac restored DA and DOPAC levels.

Discussion

Parkinson's disease is neurodegenerative disorder characterized by symptoms like tremor, rigidity, akinesia and postural instability. It is associated with a loss of dopaminergic neurons in the substantia nigra. The etiology of PD is still unknown, neuroinflammation and oxidative stress are considered as the leading cause of neurodegeneration in PD. Therefore, prostaglandins inhibitors and ROS scavenging anti-oxidants could be effective to combat progressive neurodegeneration induced by neuroinflammation in PD.

In the present study we aimed to explore the neuroprotective role of diclofenac against CPZ induced Parkinson's like symptoms. Previous researches explained that NSAIDs, especially diclofenac, play a major role in inhibiting neuroinflammation due to which it prevent the progression of PD by inhibiting inflammatory cascade (Driver et al. 2011; Ton et al. 2006). Both selective and nonselective inhibitors of cyclo-oxygenase (COX) has been reported to reduce neuronal toxicity in animal model of parkinson's disease (Choi et al. 2009, 2013). However the molecular mechanism through which NSAIDs get involved in the neuroinflammatory process and prevent neuronal loss remains unclear. Neuroinflammation is an intricate mechanism that requires several counter regulating mechanisms or inflammatory mediators like COX derived prostaglandins and other cytokines to start inflammation which results in pathological process such as Parkinson's and other neurodegenerative diseases (Griffin 2006; Reines et al. 2004). Experimental studies showed that non selective COX inhibitors, for example indomethacin, diclofenac and flurbiprofen, reduces the levels of

Table 1 Effect of diclofenac on catalepsy

Test	Time (Minute)	Groups				*P* value
		Control	Chlorpromazine 3 mg/kg/day	Diclofenac sodium 20 mg/kg/day	L-dopa/ Carbidopa 30 mg/kg/day	
Catalepsy (sec)	5	1.2 ± 0.12	11.6 ± 3.194**	9.5 ± 2.02*	5.9 ± 0.958	<0.01
	60	1.4 ± 0.158	109 ± 8.159**	57.2 ± 9.36**	72.4 ± 10.81**	<0.001
	120	1.3 ± 0.211	321.5 ± 26.47**	44.8 ± 5.37**##	35.5 ± 3.63**##	<0.001
	180	1.3 ± 0.151	183.9 ± 17.93**	14.7 ± 2.62##	12.1 ± 2.52##	0.001

Values are in $\bar{x}$ ± SEM, n = 10, df (1, 36)

*$P < 0.05$, **$P < 0.01$ as compared to control group,

# $P < 0.05$, ## $P < 0.01$ as compared to chlorpromazine group

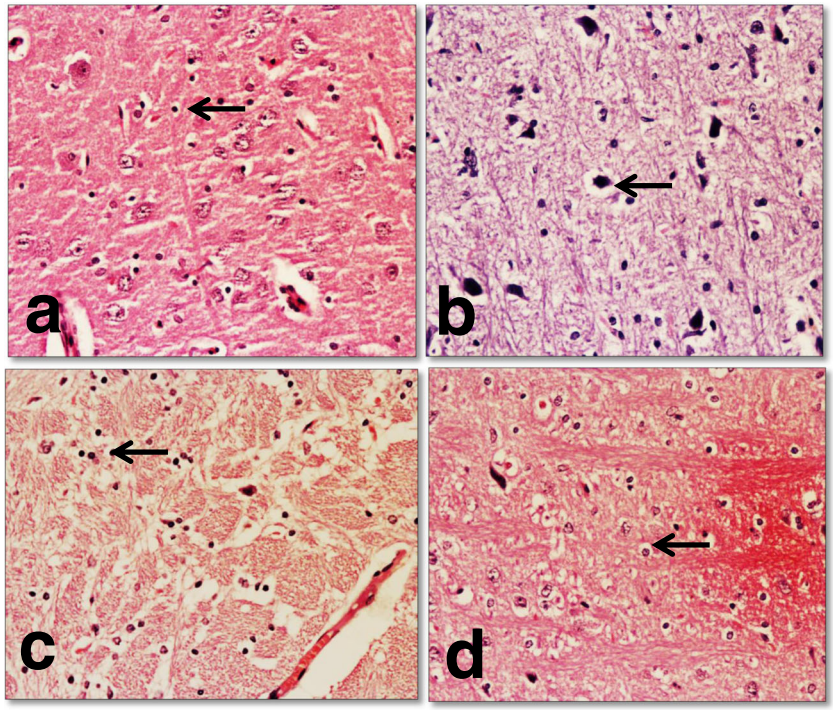

Fig. 3 Microscopic appearance of the substantia nigra stained with H & E. in Different Treated Groups. Microphotographs of H&E at ×400 HPF represents, **a** = Histopathology of control rat's brain showed normal neurons with obvious nuclei (pointed by arrows). **b** = Histopathology of CPZ treated rat's brain showing darkly stained cells have irregular shapes and condensed nuclei with empty spaces around them (pointed by arrows), some micro architecture distortion and gliosis can be seen. **c** = Histopathology of Levodopa Carbidopa treated rat's brain shows mild gliosis and edema (pointed by arrows) with few astrocytes. **d** = Histopathology of Diclofenac treated rat's brain shows proliferative blood vessels and neurons (pointed by arrows), spongy changes, mild gliosis and regeneration of neuron cells

inflammatory mediators like PGE2 and interleukin α that released by microglia and astrocytes (Al-Amin et al. 2013; Townsend and Pratico 2005). A marked increase in COX-2 has been observed both in PD patients and in mice treated with the neurotoxin MPTP (Langston et al. 1999; Yamada et al. 1992), Moreover, transgenic mice deficient in COX-2 showed a reduced degree of neurodegeneration after MPTP administration (Hoozemans and O'Banion 2005; Feng et al. 2003). Evidence of inflammation in neurodegenerative diseases suggests the possible therapeutic role of NSAIDs in treating neurodegenerative diseases.

In the present study we evaluated the neuroprotective effect of diclofenac in CPZ induced Parkinson's animal model. Certain neuroleptics especially chlorpromazine and haloperidol induce Parkinsonism by producing marked motor impairment, dystonia and other extrapyramidal effects (Kołaczkowski et al. 2014). CPZ induced catalepsy is widely used animal model of parkinson's disease (Kulkarni 2007; Sandhu and Rana 2013; Naeem et al. 2017) it produced Parkinson's like symptoms by disturbing catecholamines storage at nerve terminals and synapses resulting in reduced availability of dopamine, 5HT and noradrenaline at nerve terminals

Table 2 Effect of diclofenac on dopamine and DOPAC in cataleptic rats' brain

Brain biogenic amines	Groups				*P* value
	Control	Chlorpromazine 3 mg/kg/day	Diclofenac sodium 20 mg/kg/day	L-dopa/ Carbidopa 30 mg/kg/day	
DA ng/gm	257.72 ± 18.428	176.87 ± 29.20**	207.56 ± 11.87*	213.78 ± 14.92*	<0.001
DOPAC ng/gm	274.21 ± 26.07	232.96 ± 21.49*	256.48 ± 14.07*#	246.32 ± 19.79*#	<0.001

Values are in $\bar{x}$ ± SEM, n = 10, df (1, 36)

*$P < 0.05$, **$P < 0.01$ as compared to control group,

# $P < 0.05$, ## $P < 0.01$ as compared to chlorpromazine group

(Wakade et al. 2002; Parikh et al. 2003). CPZ deplete dopamine and its receptor in basal ganglia and in substantia nigra and induce dopaminergic neuronal death which can be seen in animal models by assessing their motor symptoms and catatonia (Sandhu and Rana 2013; Kołaczkowski et al. 2014; Khatoon et al. 2016; Shiozaki et al. 2000). In present study we observed gradual motor deterioration by CPZ treated group on wire hanging test, open field activity and catalepsy test. CPZ in a dose of 3 mg/kg/day i.p injection significantly produced cataleptic behavior in rats as apparent by a significant increase in latency time spent on a wooden bar after 21 days as compared to control, diclofenac and standard group. All antipsychotic drugs including CPZ have potent dopamine receptor blocking capacity they reduce dopamine transmission and increased GABAergic inhibition (Taepavarapruk et al. 2000). Use of the NSAIDs in PD associated motor deterioration and neurochemical changes has not been formally evaluated before and was controversial. As Sairam et al. (2003) demonstrated that diclofenac or celecoxib do not protect against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats, however on the other hand De La Garza and Asnis (2003) explored that diclofenac sodium attenuates IFN-alpha induced alterations to monoamine turnover in prefrontal cortex and hippocampus and could be an alternative option for neuroprotection.

Supporting the previous researches (Naeem et al. 2017; De La Garza et al. 2004, 2005) in our study we discovered diclofenac significantly reverts the CPZ induced locomotor deficit and cataleptic behavior. Significant increase in locomotor activity and increase in muscle activity and grip in diclofenac treated rats could be proved its neuroprotective role. Findings of our study are in agreement with study presented by Swiatkiewicz et al. (2013) which showed that NSAIDs decreases oxidative stress by inhibiting ROS species and iNOS while increasing PPAR γ which attenuates neuronal inflammation (Ajmone-Cat et al. 2010). The protective effect of diclofenac against CPZ induced cataleptic model is suggesting that NSAIDs can play a role in the modulation of neurotransmitters.

Motor fluctuations (tremors, shuffling gate, dyskinasia and muscular rigidity) are the core diagnostic symptoms of Parkinson's disease (Aerts et al. 2012; Klockgether 2004). Muscular coordination and grip abnormalities in parkinson's patients are common due to loss of dopamine neurons in brain especially substantia nigra (Forno 1996). In this study we investigated grip strength by using wire hanging test. CPZ treated rats showed significant reduce grip strength after 10 days and even they completely lost their grip ability after 21 day of treatment due to weak muscle coordination. Our results are in accordance with terry et al. (2008) they reported that chlorpromazine or olanzapine can impair the daily performance task and muscular grip of the patients. Whereas diclofenac administration after 21 days significantly improved motor coordination in parkinson's induced rats. This is in accordance with previous researches which demonstrate that NSAID increases the levels of dopamine up to normal and decreases its toxic metabolite concentration (Hassanein et al. 2004; Makunts et al. 2018). As muscle coordination relies on dopaminergic system of motor cortex, hippocampus and basal ganglia therefore it is obvious that diclofenac may enhance concentration of dopamine in basal ganglia by following up-regulation on dopaminergic framework (Casper et al. 2000).

Histopathology of our rat's brain tissues showed that CPZ treated rats showed marked degeneration, gliosis and architecture distortion with moderate darkly stained cells lying on the surface of neurons which are indicative of oxidative stress in comparison to control group (Fig. 3). CPZ produces oxidative stress by producing inflammation in brain and leads towards tissue damage (Khatoon et al. 2016; Deavall et al. 2012). Chlorpromazine induced Parkinsonism by interfering with the storage of catecholamines in intracellular granules which may cause dopamine depletion in nerve terminals and induction of hypolocomotion and muscular rigidity (Guldberg and Yates 1969). Diclofenac after 21 days of treatment significantly ameliorate these neuronal changes by decreasing oxidative stress and neuroinflammation (Al-Amin et al. 2013; Ajmone-Cat et al. 2010) as well as prevents the CPZ induced reduction of dopamine and DOPAC levels in mid brain region of cataleptic rat's brains. De La Garza and Asnis (2003) and Kaplan et al. (2017) indicated that diclofenac significantly increases dopamine levels by modulating IFN-alpha-induced neurochemical alterations. Previous research studies on NSAIDs have shown significant antioxidant properties by producing a significant reduction in ROS and iNOS radicals (L' Episcopo et al. 2010; Conner and Grisham 1996; Asanuma et al. 2001; Díaz-González and Sánchez-Madrid 2015). Hence, any decrease in the degree of catalepsy and rise in the DA and DOPAC levels in the diclofenac treated group indicates the ability of the diclofenac to protect against the oxidative stress in the brain tissue and reduce the severity of CPZ-induced catalepsy. In conclusion we hypothesized that anti-inflammatory and antioxidant property of diclofenac may be neuroprotective in PD, by preventing neuronal death and restoring dopamine levels caused by intracellular free radicals.

Conclusion

Our results suggest that diclofenac is able to reverse the behavioral motor deficits and neuronal loss induced by CPZ and confirm that these effects may be related to its potent anti-inflammatory activity. Therefore there is a need that diclofenac may be further investigated in invivo and invitro models for its proposed use in Parkinson's disease.

Compliance with ethical standards

Conflict of interest No conflict of interest associated with this work.

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