Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.2, No.8, 2012

Morin Attenuates Haloperidol Induced Tardive Dyskinesia and Oxidative Stress in Mice

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Abstract

Antipsychotic treatment with classical neuroleptics in humans can produce a serious side effect, known as tardive dyskinesia (TD). TD, is most serious iatrogenic movement disorder, has been tentatively associated with nigrostriatal dopaminergic supersensitivity and with oxidative stress in brain region and the complete pathophysiology is still obscure. In the present study we investigated the effects of co-administration of morin (30 mg/kg b.w, for 14 days) and haloperidol (HP) (1.0 mg/kg i.p, once daily, for 14 days), as well as the effects of 14 days treatment with this dose of morin after withdrawal from HP in mice. Administration of HP led to significantly increased oxidative stress, reduced antioxidants, dopamine level and condensed behavior patterns (vacuous chewing movements (VCMs), narrow beam walking, akinesia, hang test and stride length measurement), reduced nigrostriatal dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT 2) and increase the inflammatory marker β-actin expressions. Pre-treatment with morin significantly reduces oxidative stress, improves dopamine level, ameliorate motor behavior and reversed expression of DAT, VMAT 2 and β-actin in striatum. These results indicate that morin have beneficial role in mitigating HP-induced damage of dopaminergic neurons, possibly via its neuroprotective and its antioxidant potential.

Key words: tardive dyskinesia, iatrogenic, bradykinesia, nigrostriatal, antioxidant

1. Introduction

Tardive dyskinesia (TD) is a serious neurological syndrome or extra pyramidal symptoms associated with long term treatment with neuroleptics to humans and non-human primates, characterized by repetitive involuntary movements, involving the mouth, face and tongue and sometimes, limb and trunk musculature (Tsai and Coyle, 2002; Turrone et al., 2003). This syndrome persists for months after the neuroleptic has been stopped and TD develops in a large percentage of neuroleptic treated subjects and is potentially irreversible, and the syndrome is major clinical and ethical importance (Aberg et al., 1992). Despite the awareness that neuroleptics could cause extra pyramidal symptoms, these drugs remain most effective means of treating schizophrenia and for the management of behavioral disorders in developmentally disabled individuals (Kulkarni and Naidu, 2001).

Oxidative stress and lipid peroxidative products are implicated in the etiopathology of TD (Coyle and Puttfarcken, 1993; Andreassen and Jorgensen, 2000). Treatment with neuroleptics is reported to increase free radical formation and oxidative stress (Balijepalli et al., 2001). Elkashef and Wyatt (1999) have reported that experimental animals with VCMs had significantly higher thiobarbituric acid reactive substances (TBARs) in the striatum, suggesting increased lipid peroxidation and free radical production in neuroleptics treated animals. Specifically, neuroleptic drugs, by blocking dopamine (DA) receptors, cause a secondary increase in the turnover and metabolism of DA, which may lead to the formation of DA quinines as well as hydrogen peroxide through activity of monoamine oxidase-B enzyme (Lohr, 1991) in the drug metabolism. Anti-dopaminergic drugs tend to suppress the behavioral manifestations of TD, whereas dopaminergic agonists exacerbate the syndrome (Tarazi et al., 2001). The free radicals products derived from the metabolism of DA and/or from an enhancement of the glutamatergic transmission, secondary to presynaptic DA receptors blockage has gained experimental support in the literature (Naidu and Kulkarni, 2001). However, specific neurochemical markers for the disease include a profound reduction in the titers of DA, as well as levels of tyrosine hydroxylase and the DA transporter (Heikkila and Sonsalla, 1992) and vesicular monoamine transporter 2 (Miller et al., 1999).

Flavonoids are considered as valuable therapeutic agents in modern medicine and are used as herbal medicines in traditional systems, which exhibit various pharmacological activities (Fang et al., 2003). Previous studies have reported morin (3, 5, 7, 2, 4 -pentahydroxyflavone), belongs to flavonoid family found in many medicinal plants

(Aggarwal and Shishodia, 2006). It possess various pharmacological and biochemical effects including anti-oxidation, anti-mutagenesis, anti-inflammation (Fang et al., 2003), anti-neoplastic, cardioprotective, (Middleton et al., 2000), anti-cancer (Brown et al., 2003), xanthine oxidase inhibition (Yu et al., 2006), protein kinase C activation (Cao et al., 2006) and cell proliferation inhibition (Kuo et al., 2007). Morin modulated the activities of the drug metabolic enzymes, including cytochrome P450 (Hodek et al., 2003) and it protected various human cells, like myocytes, endothelial cells, hepatocytes and erythrocytes, against oxidative damages (Kitagawa et al., 2004). The potent neuroprotective activity of morin against experimental ischemia could be of therapeutic value for the treatment of acute neuronal damage and disability (Gottlieb et al., 2006). However, more data are needed to assess the true impact of morin on neurodegenerative disease.

2. Materials and methods

2.1. Animals

Adult Swiss Albino male mice (4-6 weeks, 20-30 gm) were procured from Central Animal House, Raja Muthaih Medical College, Annamalainagar. The mice were kept under controlled conditions of temperature (22±1 $^{\circ}$C), humidity (60±5%), and illumination (12h light; 12h darkness) in animal house. The animals were allowed free access to food and water. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Science Academy for the use and care of experimental animals (Animal Ethical Approval No: 671/22.09.2009).

2.2. Drug and treatment schedule

A pilot study was conducted with three different doses of morin (15, 30 and 45 mg/kg) to determine the dose dependent effect of morin in HP induced TD in mice. It was observed that after the experimental period of 14 days, morin pretreatment at the doses of 15, 30 and 45 mg/kg bw appreciable enhancement in the levels of DA in HP induced mice. From the results it was observed that 30 and 45 mg/kg bw of morin administration showed similar induction in DA levels but 15 mg/kg is very less significant. As consequence, we have chosen the optimum dose (30mg/kg) for our acute study.

HP and morin were purchased from Sigma-Aldrich chemical Company (St Louis, MO, USA). All other chemicals used in the study were of analytical grade. HP and morin were dissolved separately in normal saline solution (0.9% NaCl, pH 7.2). HP was prepared fresh every day of administration. 24 mice were randomly divided into four groups: the first group (group I) of animal considered as control treated with saline; second group (group II) received HP (1 mg/kg bw., i.p) daily for 14 days (Nair et al., 2008); third group (group III) received both morin (30 mg/kg bw., orally) and HP (as group II) and fourth group (group IV) received morin (as group III) only. Behavioural assessments were done after 14th day. The mice were sacrificed by cervical dislocation after behavioral assessment to determine the biochemical and neurochemical variables.

3. Biochemical indices

3.1. Superoxide Dismutase (SOD)

Midbrain SOD activity was assayed using xanthine and xanthine oxidase, superoxide generators and nitro blue tetrasolium as a superoxide indicator. The assay mixture consisted of 960 µl of 50mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.025 mM NBT, and 0.1 mM EDTA, 20µl xanthine oxidase and 20µl of the midbrain supernatant. Changes in absorbance were observed spectrophotometrically at 560 nm. The activity was expressed as units/min/mg protein (Oberley and Spitz, 1984).

3.2. Reduced Glutathione (GSH)

The levels of GSH in the midbrain homogenate were measured by the method described by Jollow et al., 1974. Midbrain tissue homogenate was centrifuged at 16,000×g for 15 min at 40^{0}C. The supernatant (0.5 ml) was added to 4 ml of ice-cold 0.1 mM solution of 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) in 1M phosphate buffer (pH8). The yellow color optical density was read at 412 nm in a spectrophotometer.

3.3. Catalase (CAT)

The assay mixture contained 0.05 M phosphate buffer (pH 7.0), 0.019 M hydrogen peroxide and 0.05 ml of midbrain supernatant in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of µ moles of H_2O_2 consumed/min. per mg protein (Claiborne, 1985).

3.4. Lipid Peroxidation: TBARS

To 0.2 ml of homogenate, 0.4 ml of tri carboxylic acid and 0.4 ml of thio barbitutric acid was added. The reaction mixture from the vial was transferred to the centrifuge tube and centrifuged at 3500 rpm for 15 min. The supernatant was transferred to another tube and placed in a boiling water bath for 10 mins. Thereafter, the test tubes were cooled and the absorbance of the color was read at 535 nm (Utley et al., 1967).

3.5. Glutathione Peroxidase (GPx)

The assay mixture consisted of 1.44 ml phosphate buffer, 0.1 ml of EDTA, 0.1 ml of sodium azide, 0.05 ml of glutathione reductase, 0.1 ml of glutathione (1 mM), 0.1 ml of NADPH (0.2 mM), 0.01 ml of hydrogen peroxide (0.25 mM) and 0.1 ml of homogenate (10% w/v) in a final volume of 2.0 ml. The disappearance of NADPH at 340 nm was recorded at room temperature (Mohandas et al., 1984).

4. Neurochemicals

4.1. *DA*

Striatal DA was analyzed reversed phase ion-exchange chromatography combined with electro chemical detection under isocratic conditions. The mobile phase (0.6mM 1-octanesulfonic acid, 0.27 mM Na3 EDTA, 0.043M triethylamine and 35 ml acetonitrile/litre, adjusted to pH 2.95 with H_3 PO3) are delivered at a flow rate of 0.65 ml/min at 22°C onto the reversed phase column filled with Nucleosil 120-3 C18 (Knaur, Berlin, Germany). Data were calculated by an external standard calibration (Damodaran and Mohanakumar, 1998). DA, DOPAC and HVA concentration was determined from the standard curve obtained using DA (Sigma).

5. Behavior Parameters

5.1. Vacuous chewing movements

To quantify the occurrence of oral movements, mice were placed individually in observation cages (16 cm×30 cm×19 cm) and hand operated counters were employed to quantify the frequency of vacuous chewing movements, as described by Carvalho et al., 2003. VCM are operationally defined as single mouth openings in the vertical plane not directed toward physical material. If VCM occurred during a period of grooming, they were not taken into account. The incidence of this parameter was measured continuously for 4min. Tongue protrusions (TP) and facial jerking (FJ) are counted during same time. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of vacuous chewing movements when the animal was faced away from the observer.

5.2. Narrow Beam walking

Narrow beam walking is a simple test to motor coordination of the animal by allowing animals free to move on a stationary but narrow wooden beam which requires balance and equilibrium. The beam was flat (length 130 cm and width 1 cm) and was placed at a height of 100 cm from the floor to avoid the intentional falling. The mice were trained to walk in the beam from one end of the beam (Start area) to reach another end (Target area). During that, walking time to cross the beam and the number of foot errors were noted.

Animals were videotaped while traversing the beam for a total of five trials. Videotapes were viewed and rated in slow motion for errors and time to traverse across five trials by an investigator blind to the mouse group. An error was counted when, a limb (forelimb or hind limb) slipped from the beam surface (Crabbe et al., 2003; Fleming et al., 2004) during a forward movement.

5.3. Akinesia

Akinesia was measured by noting the latency in seconds (s) of the animals to move all four limbs and the test was terminated if the latency exceeded 180 s. Each animal was initially acclimatized for 5min on a wooden elevated (30 cm) platform (40 cm×40 cm) used for measuring akinesia in mice. Using a stopwatch, the time taken (s) by the animal to move all the four limbs was recorded (Muralikrishnan and Mohanakumar, 1998; Mitra et al., 1992). This exercise was repeated five times for each animal.

5.4. Hang test

Neuromuscular strength was determined by the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid (grid 12 cm^2 opening 0.5 cm^2) and supported until they grabbed the grid with both their fore and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was

mounted 20 cm above a hard surface, to discourage falling or injury in case of falling. The apparatus was equipped with a 3-inch wooden wall to prevent animals from moving to the upper side of the grid. Animals were allowed to stay on the grid for 30 s and 10 chances were given with 1 min interval and the best fall values were recorded. The percentage of success was recorded as maximum time hanging/30 sec ×100 (Mohanasundari et al., 2006).

5.5. Stride Length Measurement (Foot print analysis)

A runway (4.5 cm wide, 50 cm long with borders of 12 cm height) was arranged to lead out into a wooden box (20 × 17 cm). Stride lengths were measured by a method adapted from D'Hooge et al., (1999) by wetting animal's fore and then hind paws with commercially available black ink and allowed them to trot on a strip of paper (4.5 cm wide, 48 cm long) down the brightly lit runway towards the goal box. The forelimb stride lengths were first measured for all the animals, then the hind limbs on a new strip of paper, once the forelimb inked paws had dried. Stride length was determined by measuring manually the distance between each step on the same side of the body, measuring from the middle toe of the first step to the heel of the second step (Fernagut et al., 2002). The three longest stride lengths (corresponding to maximal velocity) were measured from each run. Pawprints made at the beginning (7 cm) and the end (7 cm) of the run were excluded because of velocity changes. An average of at least four clear steps was calculated (Tillerson and Miller 2003). Runs in which the mice made stops or obvious decelerations observed by the experimenter were excluded from analysis (Fernagut et al., 2002). The animals were immediately put back into their home cage upon their completion of the task.

6. Western blotting

Striatal sample were prepared according to the methods described by Wright et al., (1998). In brief, striatal tissue was homogenized in an ice-cold RIPA buffer (1% Triton, 0.1% SDS, 0.5% deoxycholate, 1 mmol/L EDTA, 20 mmol/L phenyl methylsulfonyl fluride (PMSF)). The homogenate was centrifuged at 12,000 rpm/min for 15 min at 4 °C to remove debris. Protein concentration was measured by the method of Lowry et al (1951). Sample containing 50 µg of total cellular protein loaded and separated on 10% SDS polyacrylamide gel electrophoresis. The gel was then transferred on to a PVDF membrane (Millipore). The membranes were incubated with the blocking buffer containing 5% non-fat dry milk powder or BSA for 2 hr to reduce non-specific binding sides and then incubated in β-actin (rabbit polyclonal; 1:500 dilution in 5% BSA in Tris-buffered saline and 0.05% Tween-20 (TBST)), β-actin (rabbit polyclonal; 1:500 dilution in 5% BSA in Tris-buffered saline and 0.05% Tween-20 (TBST)) anti mouse DA transporter (1:500) and anti mouse VMAT2 (1:1000) with gentle shaking overnight at 4 °C. After this, membranes were incubated with their corresponding secondary antibodies (anti-rabbit or anti-mouse IgG conjugated to horseradish peroxidase) for 2 hr at room temperature. The membrane was washed thrice with TBST for 30 min. protein bands were visualized by an enhanced chemiluminescence method using ECL-kit (Genscript ECL kit, USA). Bands were scanned using a scanner and quantified by image J, a public domain java image processing software, Wayne Rasband, NIH, Bethesda, MD, USA, Which of control was set to 1.

7. Data Analysis

All the data were expressed as mean ± SD of number of experiments (n = 6). Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 10.0 software and individual comparisons were obtained using Duncan's Multiple Range Test (DMRT). Values were considered statistically significant if $p < 0.05$.

8. Results

8.1. Behavior analysis

8.1.1. Vacuous chewing movements

Haloperidol (1 mg/kg b.w) treatment significantly increased the VCM, TP and FJ frequency in mice as compared to saline treated controls. Sub-acute co-administration of morin along with haloperidol suppressed the haloperidol induced VCM (Fig. 1a), TP (Fig.1b) and FJ (Fig. 1c).

8.1.2. Narrow beam walking

Figure 2 (a and b) shows the narrow beam walking test, HP treated mice (group II) were observed to have a significant increased in time duration to cross the beam and exhibit more foot slip errors compared with

control mice (group I). Morin pretreated and HP lesion mice (group III) significantly ($p<0.05$) consumed a reduced time to cross the beam and showed a decrease in the frequency of foot slip errors.

8.1.3. Akinesia

Akinesia was measured by noting the latency in seconds of the animals to move all four limbs and the test was terminated if the latency exceeded 180 s. HP treatment significantly increased the latency time in mice as compared to saline treated controls. Co-administration of morin along with haloperidol suppressed the haloperidol induced latency time compared with alone HP treated animals.

8.1.4. Hang test

Figure 4 depicts the hang test, the group II TD mice was reduced hanging time compared to the control (group I), morin pretreated and HP lesion mice (group III) showed a significant increase in the hanging time compared to HP administered mice (group II).

8.1.5. Stride Length Measurement (Foot print analysis)

Figure 5 (a and b) shows the mean stride length differences for the fore and hind limbs in the control and experimental groups. The stride length in forelimbs and hind limbs significantly decreased in the HP treated mice (group II) relative to the control mice (group I). Morin Pretreated and HP lesion (group III) showed significant increased stride length relative to the HP-intoxicated mice (group II). In all behavioral analysis there is no significant altered behavior in the HP alone pretreated (group IV) mice compared to control mice (group I).

9. Biochemical indices

Table 1 depicts the levels of TBARS and GSH and the activities of SOD, CAT, and GPx in the midbrain of the normal and experimental groups. The levels of TBARS and the activities of SOD and CAT in midbrain were significantly elevated, and the levels of GSH and the activities of GPx were significantly diminished in HP treated second group mice as compared to control. Prior administration of morin to HP treated third group mice tends to reverse the oxidative stress.

10. Neurochemical variables

The level of DA was significantly depleted in TD mice as compared to control animals. Oral administration of morin to HP exposed mice significantly improves DA level as compared to HP alone treated groups. There is no significant change absorbed in Control and morin treated groups. The DA level variation was untainted from striatal tissues of HP induced mice brain after 24hr administration (levels of DA density in µM/mg/kg) (Figure 6).

9. Western blotting

9.1. Western blotting β-actin

Western blot result showing the effects of 14 days administration of experimental animal's and control striatal sample on immunolabeling of β-actin in mice striatum. Protein samples were subjected to 7.5% polyacrylamide gel electrophoresis and transferred to PVDF membranes, which were then incubated with primary antibodies specific for β-actin, and secondary anti-mouse (for β-actin) antibody. The bands were quantified as described in experimental procedures. The ratios of optical densities of the inflammatory to that of β-actin were calculated. Bar graphs show the comparison among groups. The values are expressed as arbitrary units and given as Mean ± SD of six observations in each group. $^{\#\#}p<0.05$ compared to the control, $^{\#}p<0.05$ compared to the HP.

9.2. *Western blotting – DAT and VMAT2*

Effect of morin on the expression of neuro-transporters markers DAT and VMAT 2 in striatum of in control and experimental mice: (a). Western blotting showing Lane 1: saline treated control, lane 2: alone HP-treated, lane 4: HP + Morin-treated, lane 4: Morin treated. DAT and VMAT 2 expressions were significantly decreased following HP administration; an effect was significantly attenuated by pretreatment with Morin. The band density was quantified by densitometry analysis. Bar graphs show the comparison among groups. The values are expressed as arbitrary units and given as Mean ± SD of six observations in each group. $^{**}p<0.05$ compared to the control,$^{*}p<0.05$ compared to the HP.

10. Discussion

TD is a serious side effect caused by treatment with neuroleptic drugs. Particularly, In the present study, HP treated animals show increased frequencies of VCMs, TP and FJ as compared to saline treated control animals. Pretreatment with morin significantly reversed the HP induced VCMs, TP and FJ in sub-acute mice model of TD. Morin counteracted the increase in TP, VCMs and FJ caused by HP treatment at all the evaluation time points. Using this and other experimental models several groups of investigators have demonstrated that HP treatment (Arnaiz et al., 1999) and oral dyskinesia are closed associated with the oxidative stress process (Naidu et al., 2003; Perry et al., 2004; Singh et al., 2003) as well as neuropathological alterations within the basal ganglia (Andreassen et al., 2003) and structural alterations of dopaminergic neuronal architecture induced by HP (Marchese et al., 2002). Numerous reports indicate that an excessive production of free radicals is associated with neuroleptic use and might contribute to the onset of TD and other movement disorders, such as dystonia and Parkinsonism (Cadet et al., 1986). This effect can be related, at least in part, to a reduction in specific endogenous antioxidant mechanisms, such as a decrease in GSH and GPx levels (Shivakumar and Ravindranath, 1993) and increase the levels of antioxidant defense enzymes such as SOD and catalase (Elkashef and Wyatt, 1999). After pretreatment of morin to HP lesioned animals significantly decrease the antioxidant defense enzymes such as TBARS, SOD catalase and increase GSH, GPx and improve the orofacial abnormal movements (VCMs, FJ and TP) and behavioral patterns compared with HP alone treated animals. The narrow beam walking used to test the balance, vestibular integrity and muscular co-ordination (Fleming et al., 2004). In narrow beam walking, the HP administrated animals showed more deficits in crossing time and foot slip errors. Pretreatment of morin to HP administrated mice showed improved behavioral characteristics as evidenced by these behavior tests. Various behavioral tests such as akinesia (slowness in initiating and executing movement (Jenner, 1993), Hang test (to study the neuro-muscular strength and coordination), Stride length measurement (to study the PD gait) and etc., are used as indices to measure the motor impairments in HP or neurotoxic agents-induced behavioral abnormalities in animal models (Rozas et al., 1998). Motor disturbances are reflected by changes in behaviour patterns, at least in part, due to pathological loss of dopaminergic neurons in midbrain of the striatum with degeneration of their striatal nerve terminals. The greater the neuronal loss in midbrain lower the concentration of DA in striatum and the more severe the Parkinsonian symptoms (Bernheimer et al., 1973). The cells of the substantia nigra use DA (a neurotransmitter chemical messenger between brain and nerve cells) to communicate with the cells in another region of the brain called the stratium. Thus, a reduction in nigral DA levels results in a decrease in stratial DA that is believed to cause PD symptoms such as tremor, muscular rigidity, akinesia, and bradykinesia slowness in initiating and executing movements (Jenner, 1993). Restoration of depleted striatal DA by plant polyphenol morin may help in the restoration of behavior patterns. HP treated animals significantly increased in akinesia that means slowness in initiating and executing movement; in pretreated morin to HP treated animals showed significant improvements and reduce the movement time compared with alone HP treated animals. No significant changes were observed in saline and alone morin treated group animals. Stride length measurements that have been established as models of Parkinson's disease and like symptoms including TD gait (Fernagut et al., 2002; Tillerson et al., 2003) also showed improvement when PD mice were fed with morin. When subjected to activity tests such as narrow beam walking and foot slip error, morin fed TD mice showed Significant improvement in foot slipping errors, time taken to cross the beam was reduced and the stride length was improved. Foot printing test suggests stability in walking and coordinated movements are improved after morin treatment.

The development of TD or orofacial dyskinesia HP has been also associated with learning deficits in animal models (Rosengarten and Quartermain, 2002). In line with this, Ploeger et al., showed the involvement of mesolimbic dopaminergic activity through of the acquisition of spatial learning, impaired after the systemically (1994) injections of HP. In our results, treatment of the animals with morin extracts before haloperidol administration, showed a significant improvement in the process of spatial learning and in DA level.

Our western blot observations revealed that the expressions of DAT, VMAT2 in midbrain of the striatum were significantly reduced by HP treatment and increased inflammatory marker β-actin showing considerable striatal dopaminergic degeneration (Figs. 5 a, b, c). Hence, this study was one aspect of investigating whether the DA transporters may be involved in the development of movement disorders such as TD in patients who are treated with HP. During the present study, suggests that neurotoxic such as HP targeting the same pathway and that the apoptotic component of HP cytotoxicity is dependent on inactivation of the PI3K/Akt pathway.

The DAT, VMAT2 are abundant in DA neurons of the nigrostriatal pathway and is the entry point of several toxins including MPTP (Dauer and Przedborski, 2003). Although DAT and VMAT2 expression is essential for normal DA neurotransmission, it also renders the DA neuron susceptible to damage by toxins that can be transported by DAT. Western blot analysis indicated a decline in DAT expression and immunoreactivity in midbrain of striatum of HP treated mice. The striatum has more and heterogeneous DAT distribution and the transporter is found on plasma membranes of axon terminals (Ciliax et al., 1995). However, it is more likely that the decline in DAT protein is due to the loss of nigrostriatal fibers as a consequence of HP lessoning. The change in DAT level is in accordance with the fact that HP targets DA neuron and DAT acts as a molecular gateway for HPP^+ entry.

This study conclude the behavioral, biochemical and molecular measures as evidenced by the different types of behavior analysis and expressions of DAT, VMAT2 and β-actin respectively indicate neuroprotective effects of morin against HP induced tardive dyskinesia and dopaminergic neuronal death through oxidative stress. Hence, our findings demonstrate that phyto flavonoid morin might be regarded as powerful complementary and/or preventive therapies of TD.

Conflict of interest statement: There are no Conflict of interest.

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Table 1. Changes in the levels of SOD, CAT and GPx in control and experimental mice midbrain.

Groups	TBARS (nmoles/g)	GSH (µg/g tissue)	SOD (U^A/mg protein)	CAT (U^B/mg protein)	GPx (U^C/mg protein)
Control	1.14±0.03	0.48±.014	1.34±0.042	2.93±0.089	2.01±0.062
HP	2.31±0.07	0.18±.005	2.84±0.089	5.54±0.171	1.45±0.045
HP +Morin	1.55±0.04	0.31±0.009	1.83±0.056	3.69±0.115	1.82±0.056
Morin	1.16±0.03	0.49±.014	1.35±0.042	2.94±0.091	2.02±0.062

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

^{A}= amount of enzyme required to inhibit 50% of NBT reduction.

^{B}= µ moles of H_2O_2 consumed /min/mg protein.

^{C}= amount of glutathione utilized/minute.

Figure 1a

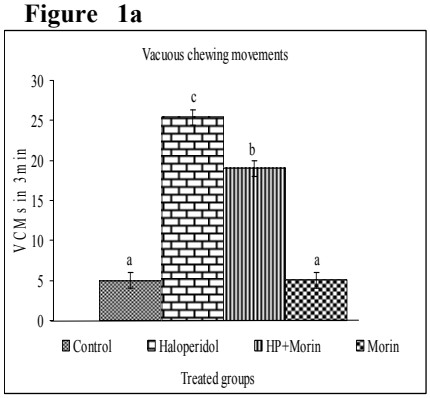
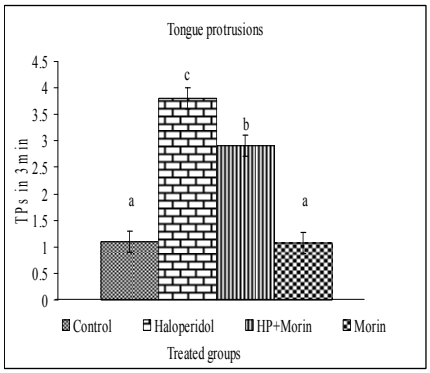


Figure 1b



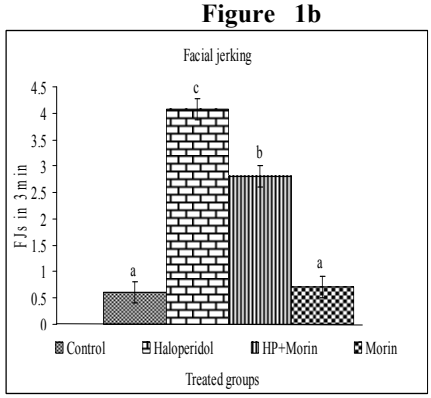


Figure 1c

Figure 1 VCM's (1a), TP (1b) and number of FJ (1c), recorded on day 15th (test day) in mice sub-acutely treated with vehicle, HP (1mg/kg, b.w, i.p. for 14 days), morin (30mg/kg) + HP (1). Data were expressed in mean ± SD. c ($p< 0.05$) as compared to control and alone morin treated group, b ($p< 0.05$) as compared to HP group.

Figure 2a

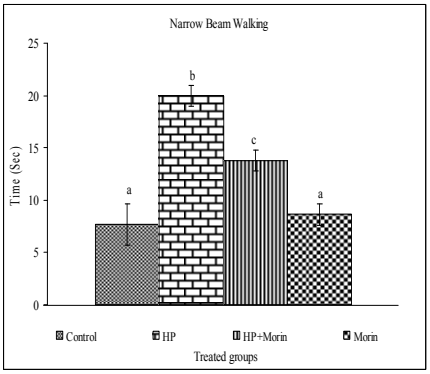


Figure 2b

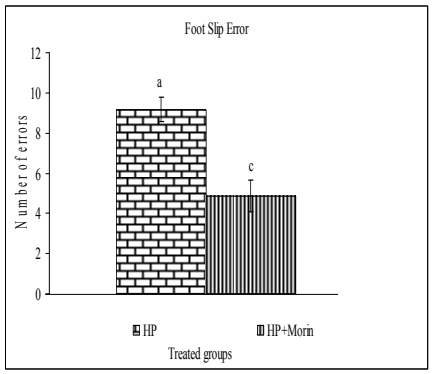


Figure 2 (a and b) depicts the narrow beam walking and foot slip errors test, the time consumption to cross the beam and foot slip errors increased in TD mouse group II compared to control mice group I. Morin pretreatment to group III significantly reduced time consumption to cross the beam and foot slip errors compared to TD mouse ($p< 0.05$).

Figure 3

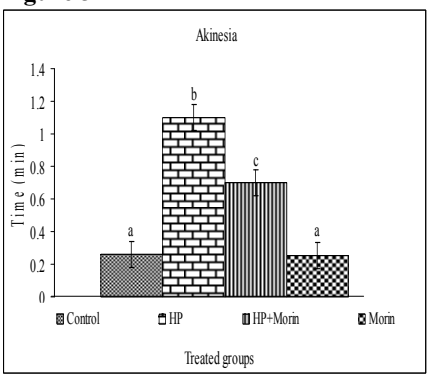


Figure 3 elucidates the akinesia test, the group II TD mice was take more time to take decision compared to the control (group I) ($p<0.05$), morin pretreated and HP lesion mice (group III) showed a significant decrease in the decision taking time compared to HP administered mice (group II) ($p<0.05$).

Figure 4

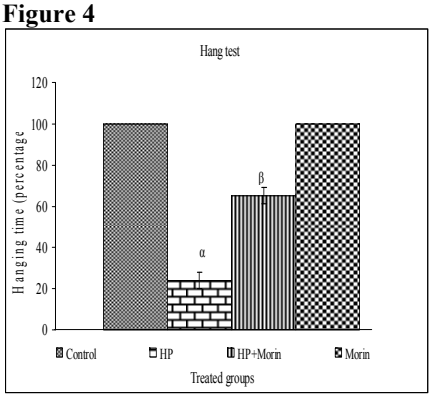


Figure 4 depicts the hang test in control ($p<0.05$), TD and morin treated mice groups and the betterment in neuromuscular coordination and strength significantly in morin pretreated group III compared to TD mice group II ($p < 0.05$).

Figure 5a

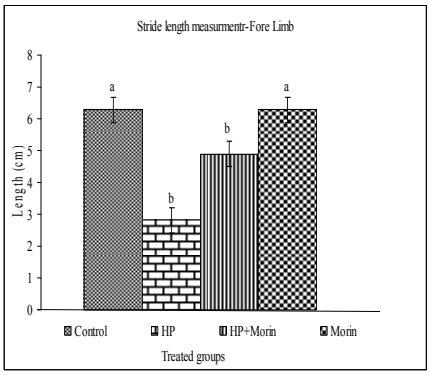


Figure 5b

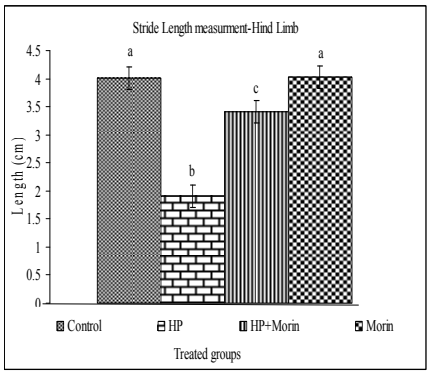


Figure 5 (a and b) Elucidate the changes in the forelimbs and hind limbs stride length in control and experimental mice groups. Group II TD mice showed reduced stride length in both forelimb and hind limb stride length measurements. Morin pretreated group III mice shown increase stride length in both forelimb and hind limb measurements ($p<0.05$).

Figure 6

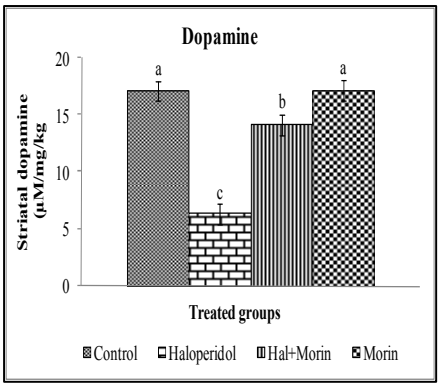


Figure 6 depicts the level of DA in control and experimental animals (levels of DA density in µM/mg/kg).

Figure 7a

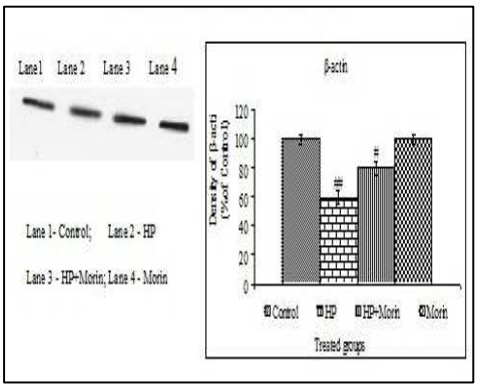


Figure 7b

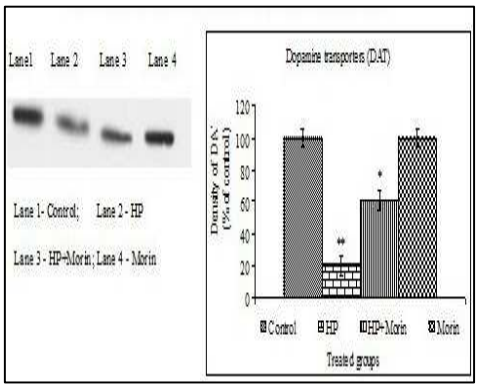


Figure 7c

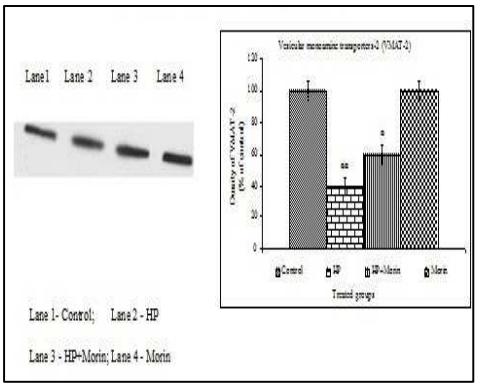


Figure 7 (a, b and c) Western blot analysis of inflammatory marker β-actin, neuro-transporters DAT and VMAT2 in striatal of control and experimental mice: DAT and VMAT2 expressions were significantly reduced following HP administration, an effect that was significantly reduced by pre-treatment with morin. The band density was quantified by scanning densitometry. Bar graphs show the comparison among groups. The values are expressed as arbitrary units and given as mean±SD of three observations in each group. $P<0.05$ compared to the control, $P<0.05$ compared to the HP control.

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