Caffeine inhibits Levodopa-Induced Potentiation of Haloperidol-Induced Catalepsy and Tardive Dyskinesia in Balb/ C Mice

Premsaroj B. Bhansali1, Shivraj Vhanale2, Sanjay Kasture3
1. Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Nashik, India;
2. Sanjivani College of Pharmaceutical Education & Research, Kopargaon, India
3. Pinnacle Biomedical Research Institute, Bhopal, India

ABSTRACT
Caffeine is a nonspecific adenosine receptor antagonist useful in treatment of Parkinson’s disease (PD). Haloperidol induced catalepsy and vacuous chewing movements in laboratory animals are inhibited by caffeine. There is a report that repeated administrations of levodopa produced increased formation of 6-OHDA in Balb/C mice which aggravates the extrapyramidal effects of neuroleptics. Chronic administration of levodopa is reported to alter mitochondrial respiratory chain activity in rats and this activity is thought to be related to an oxidative stress and is responsible for progression of PD. We therefore studied the effect of repeated administration of levodopa (with carbidopa) on haloperidol induced catalepsy and vacuous chewing movement (VCMs) and the modifications by caffeine. We also studied effect of chronic levodopa on SOD, Catalase, lipid peroxidation, and reduced glutathione levels in mice. In the experiment to study the effect of levodopa + carbidopa (LD+CD; 6:0.6 mg/kg i.p.), with or without caffeine (10 & 20 mg/kg i.p.), administered for 27 days and the duration of catalepsy in 5 min interval was recorded every 30 min till 180 min. The effect on the biochemical parameters was assessed on the 27th day. Haloperidol induced catalepsy and VCMs intensified gradually and simultaneous treatment with LD + CD potentiated both catalepsy and VCMs. Caffeine in both the doses significantly reduced duration of catalepsy and number of VCMs in all the treated groups. Caffeine also reversed the effect of haloperidol given with or without LD+CD on the enzyme markers of the stress significantly. The results suggest involvement of oxidative stress in exaggeration of pharmacological effects of haloperidol and their inhibition by caffeine. Keywords: Caffeine, haloperidol, catalepsy, vacuous chewing movements, stress

*Corresponding Author Email: drpremsarojbhansali@rediffmail.com
Received 10 April 2018, Accepted 15 May 2018
INTRODUCTION

Most patients of Parkinson’s disease (PD) receiving levodopa initially experience relief from symptoms but with prolonged use suffer various complications such as dyskinesias and variations in the antiparkinsonian response (Marsden and Parkes, 1977). Alexander et al., (1997) suggested that levodopa-induced toxicity is related primarily to DA production rather than oxidation of levodopa to toxic metabolites. Bordet et al., (2000) showed that repeated use of levodopa leads to behavioral sensitization in hemiparkinsonian rat. Asanuma et al., (2005) reported that levodopa increased dopamine turnover in parkinsonian striatum but not in the normal striatum in 6-hydroxydopamine (6-OHDA) lesioned rats. They also showed that pramipexole prevented dopaminergic neuronal damage induced by excess dopamine or levodopa. Ishida et al., (2000) reported that repeated administration of levodopa enhanced generation of hydroxyl radicals in rat striatum denervated with 6-OHDA. In line with this observation, Golembiowska et al., (2009) observed that acute as well as chronic (14 days) treatment with A2A antagonists, used as a supplement of levodopa (L–DOPA) therapy, reduced L-DOPA induced hydroxyl radical formation in striatum. There is a report that repeated administrations of levodopa caused formation of 6-OHDA in Balb/C mice (Borah and Mohankumar, 2009). Borah & Mohanakumar (2007) have also reported earlier that long-term L-DOPA treatment causes indiscriminate increase in dopamine levels at the cost of serotonin synthesis in discrete brain regions of rats.

Although the exact mechanism involved in neurodegeneration is not yet known, preclinical studies show that adenosine A2A receptors are responsible for degeneration of nigrostriatal dopaminergic neurons (Chen et al., 2001, Ikeda et al., 2002). These studies suggest usefulness of A2A receptor antagonist in treatment of PD. Epidemiological studies have shown that caffeine consumption reduces risk of PD. Joghataie et al., (2004) showed that caffeine protects against dopaminergic neuron loss and associated behavioural changes in the 6-OHDA rat model of PD. Xu et al., (2002; 2006) showed neuroprotective effect of caffeine in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD in mice without developing tolerance to neuroprotective effect on chronic treatment.

Inhibition of reserpine/ haloperidol-induced catalepsy has been widely used in preliminary evaluation of antiparkinsonian agents. Several studies have used haloperidol-induced catalepsy to understand motor symptoms associated with PD. Kobayashi et al., (1997) reported that levodopa inhibits haloperidol-induced catalepsy. Ferre et al., (1997) have proposed interaction
between D2 and A2A receptors in striatopallidal neurons responsible for inhibition of haloperidol-induced catalepsy. It is well known that neuroleptic-induced catalepsy intensifies context-dependently upon repeated testing (Klein & Schmidt, 2003; Schmidt & Beninger, 2006). Repeated administration of haloperidol induces tardive dyskinesia, which is inhibited by antioxidants (Singh et al., 2003). Luthra et al., (2009) have shown antagonism of haloperidol-induced swim impairment in levodopa and caffeine treated mice and proposed this antagonism for evaluation of drugs used in PD.

Oxidative stress and products of lipid peroxidation are suggested to be responsible for causing dyskinesia. Chronic treatment with neuroleptics increases free radical production and oxidative stress (Balijepalli et al., 2001). Both reserpine and haloperidol induce oxidative stress in striatum (Bilska et al., 2007; Manuela et al., 2004). Administration of single dose of haloperidol to mice leads to increase in oxidized glutathione (GSSG) levels in the striatum indicating generation of oxidative stress (Cohen & Spina, 1988). Elkashef & Wyatt (1999) observed that rats with vacuous chewing movement had significantly higher thiobarbituric acid reactive substances (TBARS) in the striatum, suggesting increased lipid peroxidation and free radical production in these animals. It has been hypothesized that striatal neurodegeneration caused by excitotoxic mechanisms and oxidative stress may play an important role in the development of tardive dyskinesia. Neuroleptics increase striatal glutamatergic activity in rats, which may lead to toxic effects in the striatum. Drugs that block excitotoxicity inhibit the development of persistent oral dyskinesia in the rat model. Furthermore, markers of increased oxidative stress and glutamatergic neurotransmission have been found in the cerebrospinal fluid of patients with tardive dyskinesia (Andreassen & Jørgensen, 2000; Galili-Mosberg et al, 2000). Chronic use of neuroleptics is also reported to cause decrease in the activity of antioxidant defense enzymes superoxide dismutase (SOD) and catalase (Cadet et al., 1987). Thaakur & Himabindhu (2009) have reported involvement of reactive oxygen species in haloperidol-induced tardive dyskinesia in rats. Blanchet et al., (2012) have reviewed various animal models of TD.

Since levodopa undergoes oxidative metabolism, it might accelerate the rate of nigral degeneration. Most in vivo studies do not show evidence of levodopa toxicity. However, the potential for levodopa to be toxic has not been tested under conditions of oxidative stress such as exist in PD (Mytilineou et al., 2003). Mytilineou et al., (2003) have reported that antioxidants protect from levodopa toxicity. Golembiowska et al., (2009) have demonstrated generation of hydroxyl radicals by levodopa. In view of this background information, we investigated effect of repeated administration of levodopa on haloperidol-induced catalepsy and tardive dyskinesia in
Balb/c mice and its modification by caffeine. We also studied effect on super oxide dismutase (SOD), catalase, glutathione (GSH) and lipid peroxidation (LPO) in mice treated chronically with haloperidol.

MATERIALS AND METHOD

Animals
Balb/c mice (25-30 g) obtained from National Toxicology Centre, Pune (INDIA), were used for the study. Mice were housed in colony cages and maintained at 25 ± 2°C, 12 hours light/dark cycle and 50 ± 5% relative humidity (RH) with free access to food and water. Food but not water was withdrawn 3 hr before the experiment. All the experiment carried out during the light period (8.00-16.00 hour). The studies were carried out in accordance with the guidelines given by CPCSEA, Animal Ethics committee (IAEC) (IAEC Reference Number 1093/A/07/CPCSEA).

Drugs
Levodopa (L-DOPA) and carbidopa were obtained from Smruthi Organics Limited, Solapur as gift sample. Caffeine was a gift from Glenmark pharmaceuticals, Mumbai, India and haloperidol (Serenace inj, Searle India) was purchased.

Methods

Haloperidol induced catalepsy and dyskinesia
Mice divided in four groups, each containing five received vehicle or Levodopa: carbidopa (6: 0.6 mg/kg i. p.) with or without caffeine (10 or 20 mg/kg i. p.) for 21 days. All mice received haloperidol (1 mg/kg i. p.) from day 6 till day 27. The duration of catalepsy was measured on 11th, 16th, 21st, and 27th day at 30 min interval for 180 min as described earlier (Ferre et al., 1990).

In another set of animals that received similar treatments, the number of vacuous chewing movements (VCMs) was recorded as described by Neisewander et al., (1994). The VCMs were recorded on day 11, 16, 21, and 27 for 5 min, 30 min after haloperidol. The VCMs were referred to as single mouth openings in the vertical plane not directed toward physical material. Counting was stopped whenever the mouse began grooming and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal faced away from the observer.

Biochemical Estimations

Dissection and homogenisation
On the 28th day(22nd day after receiving haloperidol), the animals were sacrificed by decapitation.
immediately after behavioural assessments. The brains were removed; cortex dissected out, rinsed with isotonic saline, and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4), the post nuclear fraction for catalase assay was obtained by centrifugation of homogenate at 1000 g for 20 min, at 4°C and for other enzyme assays centrifuged at 12,000 g for 60 min at 4°C.

**Measurement of superoxide dismutase activity**

The assay of superoxide dismutase (SOD) was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome (Misra & Fridovich, 1972; Saggu, Cooksey, & Dexter, 1989). In brief, to 0.05mL supernatant, 2.0 mL of carbonate buffer and 0.5mL of EDTA were added. The reaction was initiated by addition of 0.5mL of epinephrine and the autooxidation of adrenaline to adrenochrome at pH 10.2 was measured by following changes in optical density at 480 nm. The change in optical density every minute was measured against reagent blank. The results are expressed as units of SOD activity (milligram per protein). One unit of SOD activity induced approximately 50% inhibition of adrenaline. The results were expressed as nmol SOD U/mg wet tissue.

**Estimation of Catalase activity**

The catalase (CAT) activity assay was carried out as described by Beers and Sizer (1952). The reaction mixture consisted of 2mL phosphate buffer (pH 7.0), 0.95mL of hydrogen peroxide (0.019 M), and 0.05mL supernatant in final volume of 3 mL. Absorbance was recorded at 240nm every 10 s for 1 min. One unit of Cat was defined as the amount of enzyme required to decompose 1 mmol of peroxide per min, at 25°C and pH 7.0. The results were expressed as units of CAT activity (milligram per protein). Units of activity were determined from the standard graph of H₂O₂. The results were expressed as catalase U/mg wet tissue.

**Estimation of lipid peroxidative indices**

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehaus and Samuelsson (1968). In brief, 0.1mL of homogenate (Tris-HCl buffer, pH 7.5) was treated with 2mL of (1: 1:1 ratio) TBA–TCA–HCl reagent (Thiobarbituric acid 0.37%, 0.25N HCl, and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm. The results were expressed as LPO nanomole per milligram wet tissue.

**Estimation of reduced glutathione**

Reduced glutathione (GSH) was determined by the method of Ellman(1959). To the homogenate
10% trichloroacetic acid added and centrifuged, followed by addition of 1.0mL of Ellmанс reagent (19.8 mg of 5,50-dithiobisnitro benzoic acid (DTNB) in 100mL of 1.0% sodium citrate and 3mL of phosphate buffer (pH 8.0)). The colour developed was measured at 412 nm. The results were expressed as nanomole GSH per milligram wet tissue.

Statistical analysis

\( n = 5 \). The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. *\( p < 0.05 \) when compared with vehicle.

RESULTS AND DISCUSSION

Assessment of haloperidol induced catalepsy:

Haloperidol (1 mg/kg, i.p.) for 21 days from 6\(^{th}\) day to 27\(^{th}\) day significantly increased duration of catalepsy in vehicle treated & sub-chronic LD (6) + CD (0.6) treated mice. Sub-chronic administration of caffeine (10 & 20 mg/kg, i.p.) for a period of 27 days in mice receiving LD (6) + CD (0.6) + haloperidol significantly reduced duration of catalepsy. The observations are given in Figure 1 to 4. Further, haloperidol-induced shivering & tremors were present in vehicle treated and LD (6) + CD (0.6) treated mice while these were absent in mice that received caffeine (10 & 20 mg/kg, i.p.) along with LD (6) + CD (0.6). The duration of catalepsy increased gradually over the period of treatment, which is shown in the Fig 1 to 4.

On the 6\(^{th}\) day, mice receiving LD + CD showed prolongation of catalepsy as compared to the vehicle treated mice. Caffeine in both the doses significantly reduced the duration of catalepsy.

On the 11\(^{th}\) day, the duration of catalepsy was significantly greater in mice that received LD+CD as compared to the vehicle treated mice. Caffeine in the dose of 20 mg/kg effectively reduced the duration of catalepsy when compared with the mice that received caffeine in the dose of 10 mg/kg.

On 16\(^{th}\) day, mice receiving LD+CD showed significant increase in the duration of catalepsy at 30 and 180 min as compared to vehicle treated mice. Caffeine in the dose of 20 mg/kg was more effective than the 10 mg/kg dose.

On 21\(^{st}\) day, mice receiving LD+CD showed significant increase in the duration of catalepsy at 180 min as compared to vehicle treated mice. Caffeine in the dose of 20 mg/kg was more effective than the 10 mg/kg dose.

Assessment of vacuous chewing movements

Haloperidol (1 mg/kg, i.p.) for 21 days from 6\(^{th}\) day to 27\(^{th}\) day significantly increased VCMs in vehicle treated & sub-chronic LD (6) + CD (0.6) treated mice. Sub-chronic administration of
caffeine (10 & 20 mg/kg, i.p.) for a period of 21 days in LD (6) + CD (0.6) treated mice significantly inhibited VCMs. The observations are given in Figure 5 to 8.

On 6th day, VCMs were significantly lesser in LD+CD, caffeine (10) along with LD/CD and in caffeine (20) along with LD+CD groups. In caffeine (10) along with LD+CD, VCMs was less as compared to caffeine (20) along with LD+CD group (Fig 5).

On 11th day, VCMs are significantly increased in LD+CD treated group as compared to 6th day. In caffeine (10) and caffeine (20) along with LD+CD treated groups, no. of VCM’s were almost same, and greater than those observed on day 6 (Fig 6).

On 16th day, no. of VCMs increased in all groups as compared to 11th day. In caffeine (10) along with LD+CD treated group, the number of VCM was more as compared to those observed in caffeine (20) along with LD+CD treated group (Fig 7).

On 21st day, no. of VCMs was significantly less in both caffeine (10 & 20) along with LD+CD treated groups as compared to the other groups. In caffeine (10) along with LD+CD treated group no. of VCMs was more as compared to caffeine (20) along with LD+CD treated group (Fig 8).

Thus, the number of VCMs from fig 5 to 8, increased gradually in all the treated groups.

Biochemical Estimations

Measurement of superoxide dismutase activity

Haloperidol with or without LD+CD showed significantly lesser amount of SOD as compared to the vehicle treated group. SOD level significantly increased in both the caffeine (10 & 20 mg/kg) along with LD+CD treated groups as compared to vehicle treated group (Fig 9).

Measurement of catalase activity

The catalase activity decreased with haloperidol treatment. The catalase level was significantly increased in caffeine (20mg/kg) along with LD+CD treated group as compared to vehicle which indicates antioxidant property of caffeine.

Measurement of Lipid Peroxidase activity

Haloperidol significantly increased LPO activity in both the vehicle and LD+CD treated mice. LPO level was significantly decreased in caffeine (10) along with LD/CD and caffeine (20 mg/kg) along with LD+CD treated groups as compared to vehicle treated group.

Measurement of reduced glutathione activity

Haloperidol reduced the glutathione level significantly as compared to the vehicle treatment. LD+CD significantly increased the GSH level in mice that received LD+CD with or without caffeine and the mice that received caffeine (10) along with LD+CD showed maximum increase.
in the GSH level as compared to the mice receiving caffeine (20) along with LD+CD.

**Haloperidol induced catalepsy**

![Graph showing effect of various treatments on haloperidol induced catalepsy](image)

**Figure 1: Effect of various treatments on haloperidol induced catalepsy on day 6.**

$n = 5$, Data presented as mean ± SEM was analysed by using one-way ANOVA followed by Dunnett’s test. $* p < 0.05$

The mice receiving LD + CD showed prolongation of catalepsy as compared to the vehicle treated mice. Caffeine in both the doses significantly reduced the duration of catalepsy.

![Graph showing effect of various treatments on haloperidol induced catalepsy](image)

**Figure 2: Effect of various treatments on haloperidol induced catalepsy on day 11.** $n = 5$, Data presented as mean ± SEM was analysed by using one-way ANOVA followed by Dunnett’s test. $* p < 0.05$

On the 11th day, the duration of catalepsy was significantly greater in mice that received LD+CD as compared to the vehicle treated mice. Caffeine in the dose of 20 mg/kg effectively reduced the
duration of catalepsy when compared with the mice that received caffeine in the dose of 10 mg/kg.

![Graph showing the effect of various treatments on haloperidol induced catalepsy on 16th day.](image1)

**Figure 3:** Effect of various treatments on haloperidol induced catalepsy on 16th day

n = 5, Data presented as mean ± SEM was analysed by using one-way ANOVA followed by Dunnett’s test. * p < 0.05

Mice receiving LD+CD showed significant increase in the duration of catalepsy at 30 and 180 min as compared to vehicle treated mice. Caffeine in the dose of 20 mg/kg was more effective than the 10 mg/kg dose.

![Graph showing the effect of various treatments on haloperidol induced catalepsy on Day 21.](image2)

**Figure 4:** Effect of various treatments on haloperidol induced catalepsy on Day 21.

n = 5, Data presented as mean ± SEM was analysed by using one-way ANOVA followed by Dunnett’s test. * p < 0.05
Mice receiving LD+CD showed significant increase in the duration of catalepsy at 180 min as compared to vehicle treated mice. Caffeine in the dose of 20 mg/kg was more effective than the 10 mg/kg dose.

Figure 5: Effect of caffeine on haloperidol induced VCM’s on 6\textsuperscript{th} day in mice treated with LD+ CD.

\(n=5\). The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. \(*p < 0.05\) when compared with vehicle.

On 6\textsuperscript{th} day, VCM’s were significantly lesser in LD+CD, caffeine (10) along with LD/CD and in caffeine (20) along with LD+CD groups. In caffeine (10) along with LD+CD, VCM’s was less as compared to caffeine (20) along with LD+CD group.

Figure 6: Effect of caffeine on haloperidol induced VCM’s on 11\textsuperscript{th} day in mice treated with LD+ CD.

\(n=5\). The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. \(*p < 0.05\) when compared with vehicle.
On 11th day, VCM’s are significantly increased in LD+CD treated group as compared to 6th day. In caffeine (10) and caffeine (20) along with LD+CD treated groups, no. of VCM’s were almost same.

![Graph showing effect of caffeine on haloperidol induced VCM’s on 16th day in mice treated with LD+CD.](image1)

**Figure 7:** Effect of caffeine on haloperidol induced VCM’s on 16th day in mice treated with LD+CD. 

n=5. The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. *p < 0.05 when compared with vehicle.

On 16th day, no. of VCM’s increased in all groups as compared to 11th day. In caffeine (10) along with LD/CD treated group, no. of VCM’s was more as compared to caffeine (20) along with LD/CD treated group.

![Graph showing effect of caffeine on haloperidol induced VCM’s on 21st day in mice treated with LD+CD.](image2)

**Figure 8:** Effect of caffeine on haloperidol induced VCM’s on 21st day in mice treated with LD+CD. 

n=5. The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. *p < 0.05 when compared with vehicle.

On 21st day, no. of VCMs was significantly less in both caffeine (10 & 20) along with LD+CD treated groups as compared to the other groups. In caffeine (10) along with LD+CD treated group no. of VCMs was more as compared to caffeine (20) along with LD+CD treated group.
Biochemical Estimation:

Assessment of superoxide dismutase (SOD):

**Figure 9**: Effect of sub-chronic administration of caffeine on SOD enzyme level in haloperidol treated mice brain.

\(n=5\). The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. *\(p < 0.05\) when compared with vehicle.

Haloperidol with or without LD+CD showed significantly lesser amount of SOD as compared to the vehicle treated group. SOD level significantly increased in both the caffeine (10 & 20) along with LD+CD treated groups as compared to vehicle treated group.

**Determination of catalase level**

**Figure 10**: Effect of sub-chronic administration of caffeine on catalase level in haloperidol treated mice brain.

\(n=5\). The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. *\(p < 0.05\) when compared with vehicle.
The catalase level was significantly increased in caffeine (20) along with LD+CD treated group as compared to vehicle which indicates antioxidant property of caffeine.

**Determination of lipid peroxidation**

![Figure 11: Effect of sub-chronic administration of caffeine on lipid peroxidase level in haloperidol treated mice brain.](image)

\[ n=5. \text{ The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. } *p < 0.05 \text{ when compared with vehicle.} \]

Haloperidol significantly increased LPO activity in both the vehicle and LD+CD treated mice. LPO level was significantly decreased in caffeine (10) along with LD/CD and caffeine (20) along with LD+CD treated groups as compared to vehicle treated group.

**Determination of reduced glutathione**

![Figure 12: Effect of sub-chronic administration of caffeine on reduced glutathione level in haloperidol treated mice brain.](image)

\[ n=5. \text{ The data is expressed as mean ± SEM. One-way ANOVA is followed by Dunnett’s test. } *p < 0.05 \text{ when compared with vehicle.} \]
Haloperidol reduced the glutathione level significantly as compared to the vehicle treatment. LD+CD significantly increased the GSH level in mice that received LD+CD with or without caffeine and the mice that received caffeine (10) along with LD+CD showed maximum increase in the GSH level as compared to the mice receiving caffeine (20) along with LD+CD.

DISCUSSION

The important findings of this study showed that: a) haloperidol induced catalepsy and VCMs intensified with the repeated administrations, b) The combination of LD and CD exaggerated the effect of haloperidol, c) Caffeine significantly reduced the effect of haloperidol on catalepsy as well as VCMs, and d) Haloperidol induced changes in the biochemical markers of stress were inhibited by LD+CD and caffeine. The interaction between levodopa and caffeine seems to be dose dependent. Lucca et al., (2015) observed pharmacodynamic drug interaction between levodopa and haloperidol in PD patient. Very interestingly, an apparent enhancement of the levodopa action by the low-dose haloperidol improved the symptoms of PD and delayed the progressive increases in the L-DOPA doses, which are usually found necessary in treating Parkinson diseased patients (Hudson et al., 2014).

The inhibition of catalepsy by caffeine, observed in this study is in line with the previous observations reported by Moo-Puc et al., (2003) and Trevitt et al., (2009). Caffeine and antimuscarinic drugs act synergistically to inhibit haloperidol induced catalepsy (Moo-Puc et al., 2003) and low dose caffeine enhances usefulness of muscarinic antagonists in PD. Based on antagonism between adenosine antagonists and haloperidol, Trevitt et al. (2009) also suggested use of adenosine antagonists in treatment of PD. The reduction in haloperidol induced VCMs by caffeine observed in our study is in congruence with the observations of Bishnoi et al., (2006). Yu et al., (2006) reported cross sensitization between caffeine and levodopa in hemiparkinsonian mice using rotational behaviour as a parameter suggesting that repeated co-administration of caffeine alters levodopa responses in PD. The human studies caffeine administered before levodopa improved levodopa pharmacokinetics (Deleu et al., 2006) supporting the possibility of caffeine reducing chances of developing dyskinesia (Wills et al., 2013).

Since levodopa-induced dyskinesia are a matter of great concern, there are efforts aimed at reducing the dose of levodopa by using add-on therapies. Caffeine is the most commonly used psychoactive drug which acts as a stimulant by reducing adenosine transmission in the brain. Caffeine acts as an antagonist to both types of Adenosine A1 and A2A receptors expressed in the basal ganglia (Fisone et al., 2004). The deficit of dopamine is correlated with a complex onset of
motor symptoms (Fahn & Jankovic, 2007). Roshan et al., (2016) showed that caffeine has a potential role in the treatment of PD and have suggested need for exploring the possibilities of identifying that range of therapeutic effects using randomized clinical trials.

The mechanisms involved in haloperidol-induced catalepsy are complex and are still poorly understood. Haloperidol increases cAMP and inositol triphosphate concentration in the striatum and these effects might be due to blocking of dopamine D2 receptors (Kaneko et al., 1992). Santini et al., (2008) have indicated that persistent hyperactivation of cAMP signalling in the medium spiny neurons of the direct striato-nigral pathway play a critical role in the parkinsonian dyskinesia. Recently Kharkwal et al., (2016) revealed that the neurotransmitter acetylcholine triggers haloperidol induced catalepsy. Many studies have shown usefulness of antioxidants in reducing dyskinesia and catalepsy induced by haloperidol, however, no study has explained the mechanism involved in such an effect (Singh et al., 2003; Thaakur & Himabindhu, 2009; Lister et al., 2014). Bishnoi et al., (2006) have reported reversal haloperidol induced changes in the SOD, catalase, LPO, and GSH levels by antioxidants of plant origin. Recently, Lister et al., reported antioxidant lipoic acid reduced haloperidol induced VCMs which is in congruence with our observations. Przedborski et al., (1993) have reported that chronic administration of levodopa can cause alterations in mitochondrial respiratory chain activity in rats that are most likely related to an oxidative stress provoked by the increase in dopamine turnover.

Thus, in conclusion, levodopa + Carbidopa enhanced haloperidol induced catalepsy and vacuous chewing movements which were inhibited by caffeine. Caffeine also normalized the levels of antioxidant enzymes. Further research is necessary to understand the mechanisms involved in the potentially beneficial effect of these interactions between caffeine, levodopa and haloperidol with respect to the involvement of antioxidant enzymes.

REFERENCES

1. Andreassen Ole A., Jørgensen Hugo A. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats: Implications for tardive dyskinesia? Progress Neurobiol 2000; 61: 525-541

2. Alexander T, Sortwell CE, Sladek CD, Roth RH, Steece-Collier K. Comparison of neurotoxicity following repeated administration of l-dopa, d-dopa and dopamine to embryonic mesencephalic dopamine neurons in cultures derived from Fisher 344 and Sprague-Dawley donors. Cell Transplant. 1997; 6(3): 309-15.

3. Asanuma M, Miyazaki I, Diaz-Corrales FJ, Shimizu M, Tanaka K, Ogawa N.
Pramipexole has ameliorating effects on levodopa-induced abnormal dopamine turnover in parkinsonian striatum and quenching effects on dopamine-semiquinone generated in vitro. Neurol Res. 2005 Jul;27(5):533-9.

4. Balijepalli S., Kenchappa R.S., Boyd M.R., Ravindranath V. Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem. Int. 2001; 38: 425–35.

5. Bilska AM. Dubiel M. Sokołowska-Jez’ewicz E. Lorenc-Kocī L. Włodek Alpha-lipoic acid differently affects the reserpine-induced oxidative stress in the striatum and prefrontal cortex of rat brain. Neuroscience 2007; 146: 1758-1771.

6. Bishnoi M, Chopra K, Kulkarni SK Involvement of adenosinergic receptor system in an animal model of tardive dyskinesia and associated behavioural, biochemical and neurochemical changes. Eur J Pharmacol. 2006; 552(1-3): 55-66.

7. Bishnoi M, Chopra K, Kulkarni SK. Protective effect of rutin, a polyphenolic flavonoid against haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes. Fundam Clin Pharmacol. 2007; 21(5): 521-9

8. Blanchet Pierre J, Parent Marie-Thérèse, Rompré Pierre H, Lévesque Daniel. Relevance of animal models to human tardive dyskinesia. Behavioral and Brain Functions 2012, 8:12-20.

9. Bordet R, Ridray S, Schwartz JC, Sokoloff P. Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. Eur J Neurosci. 2000;12(6):2117-23.

10. Boulay D, Depoortere R, Oblin A, Sanger DJ, Schoemaker H, Perrault G. Haloperidol-induced catalepsy is absent in dopamine D(2), but maintained in dopamine D(3) receptor knock-out mice. Eur J Pharmacol. 2000;391(1-2):63-73.

11. Deleu D, Jacob P, Chand P, Sarre S, Colwell A. Effects of caffeine on levodopa pharmacokinetics and pharmacodynamics in Parkinson disease. Neurology. 2006; 67(5): 897-9.

12. Ferre Â, S., Fredholm, B.B., Morelli, M., Popoli, P. & Fuxe, K. (1997) Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci., 20, 482- 487.

13. Fisone G., Borgkvist A., Usiello A. Caffeine as a psychomotor stimulant: mechanism of action. Cellular and Molecular Life Sciences. 2004; 61: 857-872
14. Galili-Mosberg R, Gil-Ad, A. Weizman, E. Melamed, D. Offen. Haloperidol-induced neurotoxicity – possible implications for tardive dyskinesia. Journal of Neural Transmission; 2000; 107: 479-490

15. Gołębiowska K, Dziubina A, Kowalska M, Kamińska K. Effect of adenosine A(2A) receptor antagonists on L-DOPA-induced hydroxyl radical formation in rat striatum. Neurotox Res. 2009 Feb;15(2):155-66.

16. Hudson CJ, Seeman P, and Seeman MV. Parkinson’s Disease: Low-Dose Haloperidol Increases Dopamine Receptor Sensitivity and Clinical Response. Parkinson’s Disease. Volume 2014, Article ID 684973.

17. Lister J, Nobrega JN, Fletcher PJ, Remington G. Oxidative stress and the antipsychotic-induced vacuous chewing movement model of tardive dyskinesia: evidence for antioxidant-based prevention strategies. Psychopharmacology (Berl). 2014; 231(11): 2237-49.

18. Lister J, Ana C.Andreazza, Bushra Navaid, Virginia S.Wilson, CelineTeo, Yasika Nesarajah Alan A.Wilson, José N.Nobrega, Paul J. Fletcher, Gary Remington. Lipoic acid and haloperidol-induced vacuous chewing movements: Implications for prophylactic antioxidant use in tardive dyskinesia. Biological Psychiatry. 2017; 72: 23-9.

19. Lucca JM, Madhan Ramesh, Parthasarathi G, and Raman Rajesh. An Adverse Drug Interaction of Haloperidol with Levodopa. An Adverse Drug Interaction of Haloperidol with Levodopa. Indian J Psychol Med. 2015; 37(2): 220–2

20. Marsden CD, Parkes JD (1977) Success and problems of long-term levodopa therapy in Parkinson’s disease. Lancet 1:345-349.

21. Manuela Polydoro, Nadja Schröder, Maria Noemia M. Lima, Fábio Caldana, Daniela C. Laranja, Elke Bromberg, Rafael Roesler, João Quevedo, José Cláudio F. Moreira, Felipe Dal-Pizzol. Haloperidol- and clozapine-induced oxidative stress in the rat brain. Pharmacology Biochemistry and Behavior. 2004; 78: 751-6.

22. Moo-Puc RE, Góngora-Alfaro JL, Alvarez-Cervera FJ, Pineda JC, Arankowsky-Sandoval G, Heredia-López F. Caffeine and muscarinic antagonists act in synergy to inhibit haloperidol-induced catalepsy. Neuropharmacology. 2003; 45(4): 493-503.

23. Fahn, S., & Jankovic, J. (2007). Principles and practice of movement disorders. Philadelphia: Elsevier
25. Ishida Y, Hashiguchi H, Todaka K, Ishizuka Y, Mitsuyama Y. Repeated administration of high dose levodopa enhances hydroxyl radical production in the rat striatum denervated with 6-hydroxydopamine. Neuroscience letters 2000; 290: 33-6.

26. Chen JF, Xu K, Petzer JP, et al. Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson’s disease. J Neurosci 2001;21:RC143.

27. Ikeda K, Kurokawa M, Aoyama S, Kuwana Y. Neuroprotection by adenosine A2A receptor blockade in experimental models of Parkinson’s disease. J Neurochem 2002; 80: 262–70.

28. Kaneko M, Sato K, Horikoshi R, Yaginuma M, Yaginuma N, Shiragata M, Kumashiro H. Effect of haloperidol on cyclic AMP and inositol trisphosphate in rat striatum in vivo. Prostaglandins Leukot Essent Fatty Acids. 1992 May;46(1):53-7.

29. Mytilineou C, Walker RH, JnoBaptiste R, Olanow CW. Levodopa is toxic to dopamine neurons in an in vitro but not an in vivo model of oxidative stress. J Pharmacol Exp Ther. 2003; 304(2): 792-800.

30. Cohen G., Spina M.B. in ed. Hefti, F. & Weiner, W.J. Hydrogen production in neurones: Implications for understanding Parkinson's disease Progress in Parkinson's Research 1988. New York: Plenum; 119–126.

31. Elkashef A.M., Wyatt R.J. Tardive dyskinesia: possible involvement of free radical and treatment with vitamin E. Schizophr. Bull. 1999;25:731–740

32. Cadet J.L., Lohr J.B., Jeste D.V. Tardive dyskinesia and schizophrenic burnout: the possible involvement of cytotoxic free radicals Handbook of Schizophrenia: The Neurochemistry and Pharmacology of Schizophrenia 1987. Amsterdam: Elsevier; 425–438.438ed. Henn, F.A. & DeLisi, L.E.

33. Luthra PM, Barodia SK, Raghubir R. Antagonism of haloperidol-induced swim impairment in L-dopa and caffeine treated mice: A pre-clinical model to study Parkinson's disease. J Neuroscience Methods. 2009; 178: 284-290

34. OM AS, Youness ER, Khadrawy YA, Sleem AA. Brain and liver oxidative stress after sertraline and haloperidol treatment in mice. J Basic Clin Physiol Pharmacol. 2013; 24: 15-23.

35. Klein A, Schmidt WJ. Catalepsy intensifies context-dependently irrespective of whether it is induced by intermittent or chronic dopamine deficiency. Behav Pharmacol. 2003; 14:49-53.
36. Kharkwal G, Brami-Cherrier K, Lizardi-Ortiz JE, Sulzer D, Kreitzer AC, Borrelli B. Parkinsonism Driven by Antipsychotics Originates from Dopaminergic Control of Striatal Cholinergic Interneurons. 2016; 91: 67-78.
37. Kobayashi T, Araki T, Itoyama Y, Takeshita M, Ohta T, Oshima Y. Effects of L-dopa and bromocriptine on haloperidol-induced motor deficits in mice. Life Sci. 1997;61(26):2529-38.
38. Roshan MHK, Tambo A, and Pace NP. Potential Role of Caffeine in the Treatment of Parkinson’s Disease. Open Neurol J. 2016; 10: 42–58.
39. Santini E, Valjent Emmanuel, Fisone Gilberto. Parkinson's disease: Levodopa-induced dyskinesia and signal transduction. FEBS J. 2008; 275: 1392 – 99.
40. Schmidt, W. J., & Beninger, R. J. (2006). Behavioural sensitization in addiction, schizophrenia, Parkinson’s disease and dyskinesia. Neurotox Res, 10(2), 161–6
41. Singh A, Naidu PS, Kulkarni SK. Possible antioxidant and neuroprotective mechanisms of FK506 in attenuating haloperidol-induced orofacial dyskinesia. Eur J Pharmacol. 2003; 477(2): 87-94.
42. Thaakur Santhrani, Himabindhu G. Effect of alpha lipoic acid on the tardive dyskinesia and oxidative stress induced by haloperidol in rats. Journal of Neural Transmission 2009;116: 807-14.
43. Trevitt J, Vallance C, Harris A, Goode T Adenosine antagonists reverse the cataleptic effects of haloperidol: implications for the treatment of Parkinson's disease. Pharmacol Biochem Behav. 2009; 92(3): 521-7
44. Borah Anupom, Mohanakumar Kochupurackal P. Long term L-DOPA treatment causes production of 6-OHDA in the mouse striatum: Involvement of hydroxyl radical. Annals of Neurosciences, 2009; 16 (4)
45. Borah A, Mohanakumar KP. Long-term L-DOPA treatment causes indiscriminate increase in dopamine levels at the cost of serotonin synthesis in discrete brain regions of rats. Cell Mol Neurobiol 2007; 27:985-996.
46. Xu Kui, Xu Yuehang, Jermyn Deborah Brown-, Chen Jiang-Fan, Ascherio Alberto, Dluzen Dean E., Schwarzschild Michael A. Estrogen Prevents Neuroprotection by Caffeine in the Mouse 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Model of Parkinson’s Disease. J. Neurosci. 2006; 26(2):535–541
47. Xu K, Xu YH, Chen JF, Schwarzschild MA (2002) Caffeine’s neuroprotection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity shows no tolerance to chronic
caffeine administration in mice. Neurosci Lett 322:13–16.
49. Wills Anne-Marie A., Eberly Shirley, Tennis Marsha, Lang Anthony E., Messing Susan, Togasaki Daniel, Tanner Caroline M., Kamp Cornelia, Chen Jiang-Fan, Oakes David, McDermott Michael P., and Schwarzschild MA. Caffeine Consumption and Risk of Dyskinesia in CALM-PD. Mov Disord. 2013; 28(3): 380–3.
50. Joghataie MT, Roghani M, Negahdar F, Hashemi L (2004) Protective effect of caffeine against neurodegeneration in a model of Parkinson’s disease in rat: behavioral and histochemical evidence. Parkinsonism Relat Disord 10:465–468
51. Anupom Borah, Kochupurackal P Mohanakumar Long term L-DOPA treatment causes production of 6-OHDA in the mouse striatum: Involvement of hydroxyl radical. Annals of Neurosciences, 2009; Vol16, No. 4
52. Borah A, Mohanakumar KP. Long-term L-DOPA treatment causes indiscriminate increase in dopamine levels at the cost of serotonin synthesis in discrete brain regions of rats. Cell Mol Neurobiol 2007; 27:985-996.
53. Przedborski Serge, Vernice Jackson-Lewis, Muthane Uday, Naini AN, Fahn Stanley. Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. Annals of Neurology 1993; 34: 715–23.