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Original article:

AMELIORATION OF THE HALOPERIDOL-INDUCED MEMORY IMPAIRMENT AND BRAIN OXIDATIVE STRESS BY CINNARIZINE

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ABSTRACT

Haloperidol is a classic antipsychotic drug known for its propensity to cause extrapyramidal symptoms and impaired memory, owing to blockade of striatal dopamine D2 receptors. Cinnarizine is a calcium channel blocker with D2 receptor blocking properties which is widely used in treatment of vertiginous disorders. The present study aimed to see whether cinnarizine would worsen the effect of haloperidol on memory function and on oxidative stress in mice brain. Cinnarizine (5, 10 or 20 mg/kg), haloperidol, or haloperidol combined with cinnarizine was administered daily *via* the subcutaneous route and mice were examined on weekly basis for their ability to locate a submerged plate in the water maze test. Mice were euthanized 30 days after starting drug injection. Malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (nitrite/nitrate) were determined in brain. Haloperidol substantially impaired water maze performance. The mean time taken to find the escape platform (latency) was significantly delayed by haloperidol (2 mg/kg, i.p.) on weeks 1-8 of the test, compared with saline control group. In contrast, those treated with haloperidol and cinnarizine showed significantly shorter latencies, which indicated that learning had occurred immediately. Haloperidol resulted in increased MDA in cortex, striatum, cerebellum and midbrain. GSH decreased in cortex, striatum and cerebellum and nitric oxide increased in cortex. Meanwhile, treatment with cinnarizine (20 mg/kg) and haloperidol resulted in significant decrease in MDA cortex, striatum, cerebellum and midbrain and an increase in GSH in cortex and striatum, compared with haloperidol group. These data suggest that cinnarizine improves the haloperidol induced brain oxidative stress and impairment of learning and memory in the water maze test in mice.

Keywords: Haloperidol, cinnarizine, water maze, oxidative stress, brain

INTRODUCTION

Memory or the acquisition and recall of past experiences and learned information is fundamental to human life. Memory disorders can occur as a part of normal aging (mild cognitive impairment), in neurodegenerative diseases e.g., Alzheimer's disease and Parkinson's disease (Sawamoto et al., 2008) or psychiatric disorders e.g., major depressive illness and schizophrenia. Memory impairment can also be due to medications. In this context, antipsychotic drugs, used in the treatment of schizophrenia, have been associated with worsening of memory tasks; this being more evident with the typical antipsychotics such as haloperi-

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dol in contrast to atypical or newer generation antipsychotics which are thought to act *via* both D2 receptor antagonism and 5HT2A receptor antagonism (Beuzen et al., 1999; Gemperle et al., 2003; Houthoofd et al., 2008).

Haloperidol, a classic antipsychotic drug, is widely used to treat schizophrenic symptoms, to control agitation in patients with acute illness and delirium and also to control delusions, hallucinations, agitation, and other disruptive behavioral symptoms associated with Alzheimer's disease. The drug exerts its effect by blocking dopamine D2 receptors in the prefrontal cortex. Meanwhile, blocking striatal D2 receptors accounts for the liability of the drug to cause extrapyramidal side effects. Haloperidol impairs learning and memory performance in humans as well as in experimental animals. Thus, haloperidol impaired spatial working memory performance and planning ability in healthy volunteers (Legangneux et al., 2000; Lustig and Meck, 2005) and worsened recent autobiographical memory scores of patients with Alzheimer's disease (Harrison and Therrien, 2007). In experimental animals, haloperidol impaired memory retention in water-maze task and in step-through test (Terry et al., 2003; Hou et al., 2006; Abdel-Salam and Nada, 2011).

Cinnarizine (1-diphenylmethyl-4-(3-phenyl-2-propenyl) piperazine) is a calcium channel blocker and a vasodilator used in treatment of vertiginous disorders, cerebrovascular disturbances and in migraine prophylaxis (Pianese et al., 2002; Mansooreh et al., 2006; Desloovere, 2008). Cinnarizine has been reported to cause extrapyramidal side effects in humans (Teive et al., 2004; Hirose, 2006), primates (Garcia-Ruiz et al., 1992) and rodents (Dall'Igna et al., 2005). The inhibition of calcium influx into striatal cells and direct dopamine D1 and D2 receptor blocking properties (Reiriz et al., 1994; Brücke et al., 1995; Daniel and Mauro, 1995), inhibition of mitochondrial complexes I and II (Veitch and Hue, 1994) and inhibition of proton pumping and catecholamine uptake in storage vesicles (Terland and Flatmark, 1999) might contribute to the drug-induced parkinsonism. Cinnarizine might also impair memory performance in healthy volunteers (Golding et al., 1989**;** Parrott et al., 1990).

Oxidative stress defined as a breach in the balance between free radical production and antioxidant defense mechanisms has been implicated in the pathogenesis of neurodegenerative disorders (Mattson, 2002), depression (Sarandol et al., 2007) and schizophrenia (Behrens and Sejnowski, 2009; Bošković et al., 2011). Oxidative stress also plays an important role in the decline in cognitive functions associated with aging or caused by different pathological states such as Alzheimer's disease or Parkinson's disease. Oxidative stress and inflammatory responses underly neuronal injury and memory impairment caused by Aβ deposition in animal models of Alzheimer's disease, which could be prevented by antioxidants (Um et al., 2009; Lu et al., 2009; Dumont et al., 2009). Oxidative stress has also been associated with age related deficits in learning and memory and in cognitive impairment caused by glucocorticoids (Sato et al., 2010) or that caused by nicotine (Hritcu et al., 2011). There is also an evidence to suggest increased oxidative stress by antipsychotic drugs and in particular the typical or first generation antipsychotic drugs compared with the newer atypical antipsychotics (Dakhale et al., 2004; Pillai et al., 2007; Martins et al., 2008).

Therefore the present study aimed to: (1) investigate the effect of the classic neuroleptic drug haloperidol and the calcium channel and dopamine D2 blocking drug cinnarizine in the working memory version of MWM test and on brain oxidative stress (2) test whether treatment with cinnarizine would worsen the effect of haloperidol on memory function and oxidative stress in mice brain.

MATERIALS AND METHODS

Animals

Swiss male albino mice 20-22 g of body weight (age: 5-6 weeks) were used. Mice were obtained from animal house colony of

the National Research Centre (Cairo, Egypt). Mice were housed under standard laboratory conditions with 1 h of light-dark cycle and temperature between 24 °C and 25 °C. Standard laboratory food and water were provided *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, Revised 1985). Equal groups of 6 mice each were used in all experiments.

Cognitive testing

The maze consisted of a glass tank, narrowed to 20 cm wide, 40 cm in height, 70 cm in length, filled to a depth of 21 cm with water maintained at 25 °C. The escape glass platform was hidden from sight, submerged 1 cm below the surface of the water at the end of the tank (Dunnett et al., 2003). Haloperidol (1 mg/kg), cinnarizine (5, 10 or 20 mg/kg), haloperidol (1 mg/kg) + cinnarizine (5, 10 or 20 mg/kg) or saline was administered daily *via* the subcutaneous route and mice were examined on weekly basis for their ability to locate a submerged plate. Drugs were given 30 min before testing. Once the mice reached the platform, it remained there for 15 sec (trial 1; reference memory or acquisition trial). At the end of each trial, the mice were towel dried, returned to their home cage (where a heat lamp was available), and 3 min elapsed before the next trial (trials 2 and 3; working memory or retrieval trial), which used the same platform location and start position as trial 1. The latency to find the platform (sec) is assessed with a stopwatch.

Biochemical analysis

Mice were euthanized 30 days after starting drug injection by decapitation under ether anaesthesia, brains and livers were excised, washed with ice-cold saline solution (0.9 % NaCl), weighed and stored at -80 °C for the biochemical analyses. The liver was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10 % w/v for the biochemical assays.

Determinaion of lipid peroxidation

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the brain tissues. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al. (1994), in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

Determination of reduced glutathione

Reduced glutathione (GSH) was determined by Ellman's method (1959). The procedure is based on the reduction of Ellman´s reagent by –SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically.

Determination of nitric oxide

Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage et al. (1995) where nitrite, stable end product of nitric oxide radical, is mostly used as indicator for the production of nitric oxide.

Drugs

Haloperidol (Kahira Pharm and Chem. IND, Cairo, ARE), cinnarizine (Arab Drug Co., Cairo, A.R.E). All drugs were dissolved in isotonic (0.9 % NaCl) saline solution immediately before use. The doses of drugs used in the study were based on previous studies (Invernizzi et al., 2003; Abdel-Salam, 2007).

Statistical analysis

Data are expressed as mean ± SEM. The data were analyzed by one way ANOVA and by repeated measures (session x treatment) ANOVA, followed by Duncan's multiple range test, using SPSS software (SAS Institute Inc., Cary, NC). A probabil-

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ity value of less than 0.05 was considered statistically significant.

RESULTS

As shown in Figures 1 and 2, treatment with haloperidol substantially impaired water maze performance. The mean time taken to find the escape platform (latency) was significantly delayed by haloperidol (1 mg/kg) on weeks 1-8 compared with saline-treated control group. Cinnarizine administered at doses of 5, 10 or 20 mg/kg did not alter escape latency. Moreover, the administration of cinnarizine with haloperidol did not worsen escape latencies. On the contrary, mice treated with haloperidol and cinnarizine showed significantly shorter latencies, compared with the haloperidol only-treated group, which suggested that memory was improved by the drug treatment (Figures 1, 2).

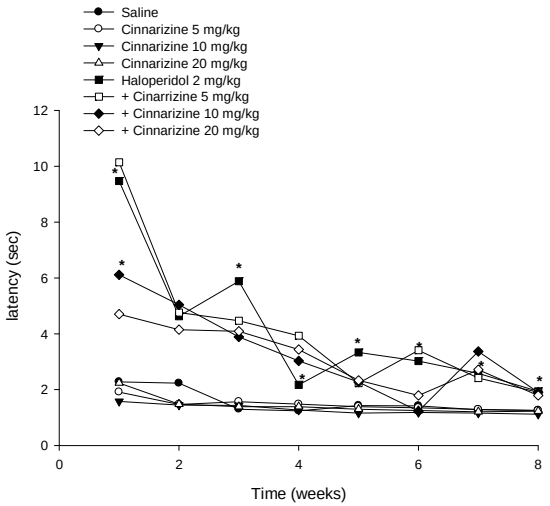

Figure 1: Effect of cinnarizine, haloperidol or cinnarizine combined with haloperidol on the latency to find hidden platform in the MWM test. Drugs were administered daily via subcutaneous route for one month and observations were done on weekly basis. Asterisks indicate significant change from the saline control group.

The mean time to reach the platform over 8 weeks for the saline (group 1), cinnarizine (5, 10 or 20 mg/kg) (groups 2-4), haloperidol only (group 5), haloperidol + cinnarizine (5, 10 or 20 mg/kg) (groups 6-8) was 1.54 ± 0.05, 1.46 ± 0.04, 1.31 ± 0.03, 1.29 ± 0.03, 4.13 ± 0.38, 4.14 ± 0.44, 3.33 ± 0.24, 3.12 ± 0.24, respectively. There was a significant main drug effect (F = 20.3, p = 0.0001), a significant main effect of weeks (F = 2.99, p = 0.0001), but no significant drug x weeks interaction or trial x weeks interaction. All groups showed progressively shorter time to locate the hidden plate improved over 8 weeks. The groups treated with haloperidol alone or haloperidol + cinnarizine 5 mg/kg showed significantly longer time to locate the hidden platform compared with all other groups.

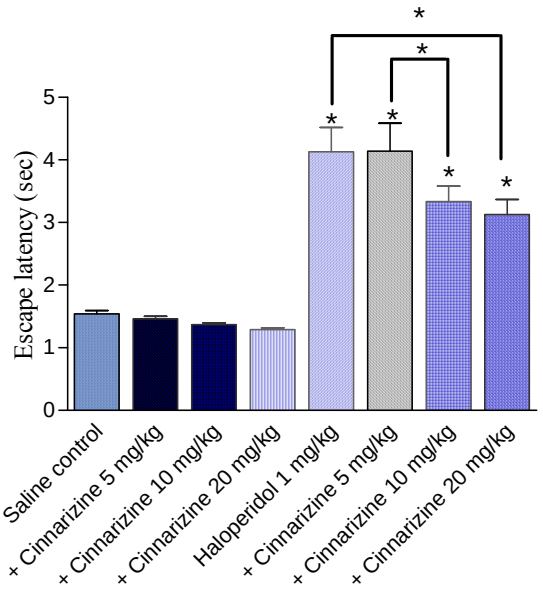

Figure 2: The average mean latency ± SEM to locate a submerged plate in the MWM test over four weeks. Mice received daily injections of saline, cinnarizine, haloperidol or cinnarizine + haloperidol and were tested weekly. Asterisks indicate significant change from the saline control group and between different groups as indicated in the figure.

A repeated measure ANOVA was done to compare latency to find the hidden plate in the first, second, and third trial across 8 weeks. In the first trial, the mean time to reach the platform over 8 weeks for the saline (group 1), cinnarizine (5, 10 or 20 mg/kg) (groups 2-4), haloperidol only (group 5), haloperidol + cinnarizine (5, 10 or 20 mg/kg) (groups 6-8) was 1.52 ± 0.08, 1.37 ± 0.06, 1.45 ± 0.07, 1.53 ± 0.16, 4.76 ± 0.51, 4.78 ± 0.32, 3.86 ± 0.26, 3.18 ± 0.33, respectively. On the first trial there was a significant main effect of drug (F = 13.51, p= 0.0001), a significant main effect of

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weeks (F = 5.96, p= 0.0001) but no significant drug x weeks interaction. Comparing different groups in trial 1 indicated that cinnarizine at 10 or 20 mg/kg with haloperidol significantly decreased escape latency when compared with the haloperidol only treated group. This suggested that the impairment in memory retention observed in the haloperidol-treated *vs.* saline group was improved by the high dose of cinnarizine. The mean time to reach the platform on the acquisition trials was also decreased by cinnarizine at 10 or 20 mg/kg when compared with the haloperidol only group (Figure 3A).

In the second trial the mean time to reach the platform over 8 weeks for the saline (group 1), cinnarizine (5, 10 or 20 mg/kg) (groups 2-4), haloperidol only (group 5), haloperidol + cinnarizine (5, 10 or 20 mg/kg) (groups 6-8) was 1.46 ± 0.06, 1.54 ± 0.22, 1.24 ± 0.03, 1.17 ± 0.03, 3.57 ± 0.32, 3.78 ± 0.28, 2.95 ± 0.23, 2.92 ± 0.24, respectively. There was a significant main effect of drug (F = 24.6, p= 0.0001) and a significant main effect of weeks (F = 11.16, p= 0.001) (Figure 3B).

In the third trial the mean time to reach the platform over 8 weeks for the saline (group 1), cinnarizine (5, 10 or 20 mg/kg) (groups 2-4), haloperidol only (group 5), haloperidol + cinnarizine (5, 10 or 20 mg/kg) (groups 6-8) was 1.64 ± 0.11, 1.8 ± 0.02, 1.18 ± 0.03, 1.32 ± 0.02, 4.06 ± 0.38, 3.85 ± 0.37, 3.19 ± 0.29, 3.28 ± 0.26, respectively. There was a significant main effect of drug (F = 15.0, p= .0001), a significant main effect of weeks (F = 10.25, p= .0001) and a significant drug x weeks interaction (F = 1.57, p = 0.01) (Figure 3C).

Figure 3 (A-C): The average mean latency ± SEM of first (A); second (B) and third (C) trial to locate a submerged plate in the MWM test over four weeks. Mice received daily injections of saline, cinnarizine, haloperidol or cinnarizine + haloperidol and were tested weekly. Asterisks indicate significant change from the saline control group and between different groups as indicated in the figure.

Fig. 3A

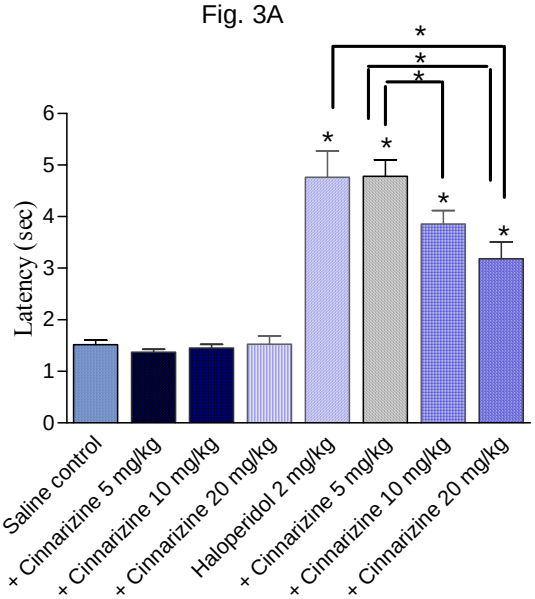


Fig. 3B

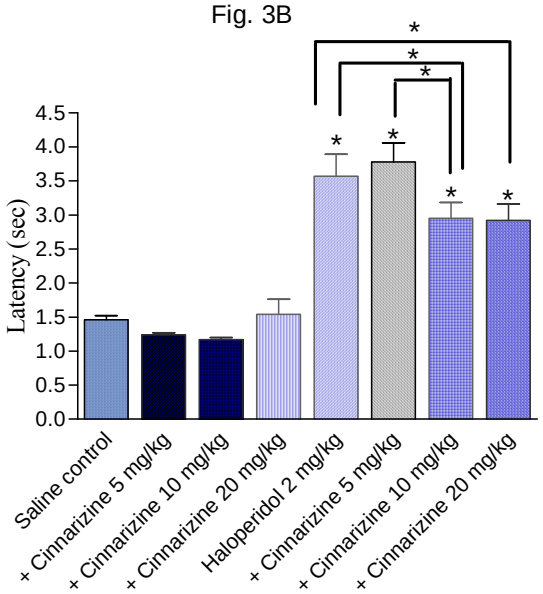
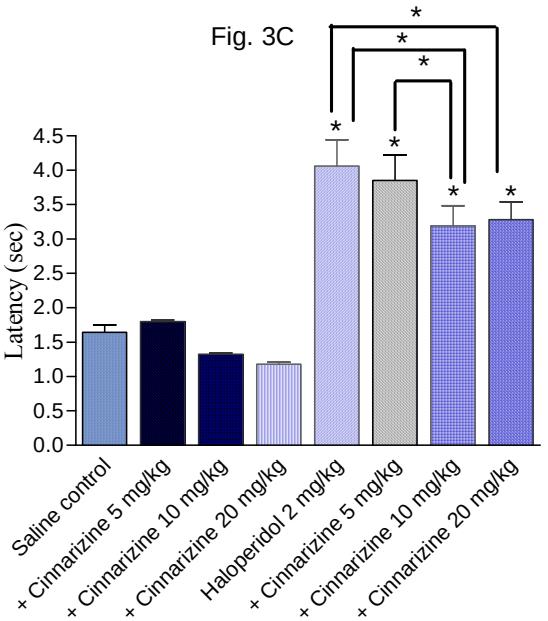


Fig. 3C



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Lipid peroxidation

In haloperidol only treated mice, there was a significant increase in lipid peroxidation in the cortex by 87.5 % (46.7 ± 3.4 *vs* 24.9 ± 1.3 nmol/g, $p<0.05$), striatum by 47.3 % (44.5 ± 2.3 *vs* 30.2 ± 1.5, nmol/g, $p<0.05$), cerebellum by 24.0 % (41.3 ± 2.1 *vs* 33.3 ± 1.4 nmol/g, $p<0.05$) and midbrain by 13.0 % (41.3 ± 2.1 *vs* 33.3 ± 1.4 nmol/g, $p<0.05$), compared with the saline-treated group. Cinnarizine alone did not alter MDA in cortex, striatum or cerebellum. Cinnarizine at 20 mg/kg decreased MDA in midbrain by 19.6 % ($p<0.05$) compared with the saline group (Figures 4A-B). Mice treated with haloperidol and cinnarizine at 10 mg/kg showed decreased MDA in cortex and striatum by 17.3 % ($p<0.05$) and 20.0 % ($p<0.05$), respectively, compared with haloperidol control value. With cinnarizine being given at 20 mg/kg with haloperidol, there was 30.8, 25.4, 24.2 and 19.2 % decrease in MDA in cortex (32.3 ± 1.4 *vs* 46.7 ± 3.4 nmol/g, $p<0.05$), striatum (33.2 ± 1.6 *vs* 44.5 ± 2.3 nmol/g, $p<0.05$), cerebellum (31.3 ± 1.7 *vs* 41.3 ± 2.1 nmol/g, $p<0.05$) and midbrain (30.8 ± 1.2 *vs* 38.1 ± 1.4 nmol/g, $p<0.05$), compared to haloperidol only control value (Figures 4A-B).

Fig. 4A

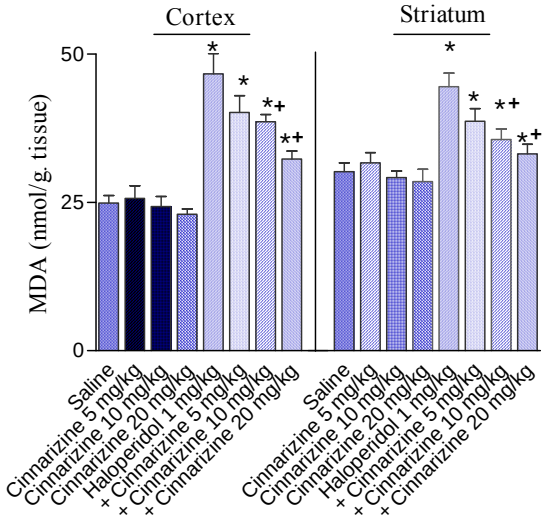

Fig. 4B

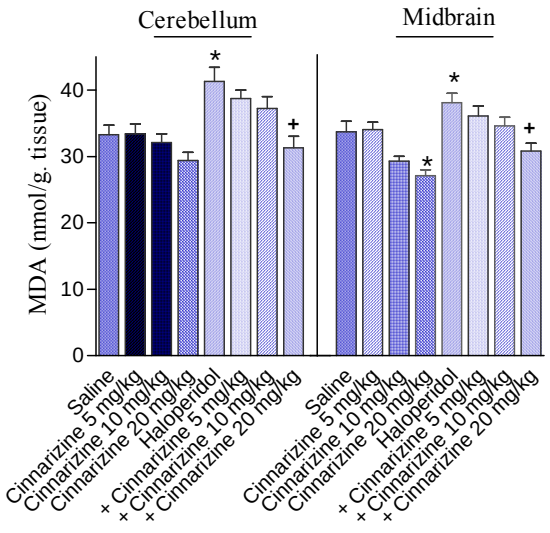

Figure 4A, B: Malondialdehyde (MDA) in cortex and striatum (A) and in cerebellum and midbrain (B) in mice treated with cinnarizine, haloperidol or cinnarizine + haloperidol for one month. *: P< 0.05 vs corresponding saline control value. + : P< 0.05 vs. haloperidol only-treated group

Reduced glutathione

The administration of haloperidol resulted in a significant decrease of reduced glutathione (GSH) ($p<0.05$) by 25.6 %, 30.6 % and 25.7 % in the cortex (7.4 ± 0.31 *vs* 9.95 ± 0.20 µmol/g, $p<0.05$), striatum (6.8 ± 0.22 *vs* 9.8 ± 0.54 µmol/g, $p<0.05$) and cerebellum (7.5 ± 0.22 *vs* 10.1 ± 0.41 µmol/g, $p<0.05$), respectively compared with the saline-treated group. A non-significant decrease in GSH by 11.6 % was registered in the midbrain (9.9 ± 0.66 *vs* 11.2 ± 0.70 µg/g, $p>0.05$). No-significant effect on GSH was observed in cinnarizine only-treated mice. However, mice treated with cinnarizine (5, 10 or 20 mg/kg) and haloperidol showed significant increase by 14.9 %, 16.2 % and 23 % in GSH in the cortex as compared to haloperidol only-treated group (8.5 ± 0.10, 8.6 ± 0.18 and 9.1 ± 0.21 *vs* 7.4 ± 0.31 µmol/g, $p<0.05$). Mice treated with cinnarizine 20 mg/kg and haloperidol showed significant increase by 18.2 % in the striatum compared to haloperidol only-treated group (8.04 ± 0.34 *vs* 6.8 ± 0.22 µmol/g, $p<0.05$). Moreover,

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mice treated with cinnarizine 20 mg/kg and haloperidol showed significant increase by 18.7 % in the cerebellum compared to haloperidol only-treated group (8.9 ± 0.31 *vs* 7.5 ± 0.22 µmol/g, $p<0.05$). A non-significant increase in GSH by 12.6 % was observed in the midbrain in mice treated with cinnarizine 20 mg/kg and haloperidol compared with the haloperidol only treatment group ($p>0.05$) (Figures 5A-B).

Fig. 5A

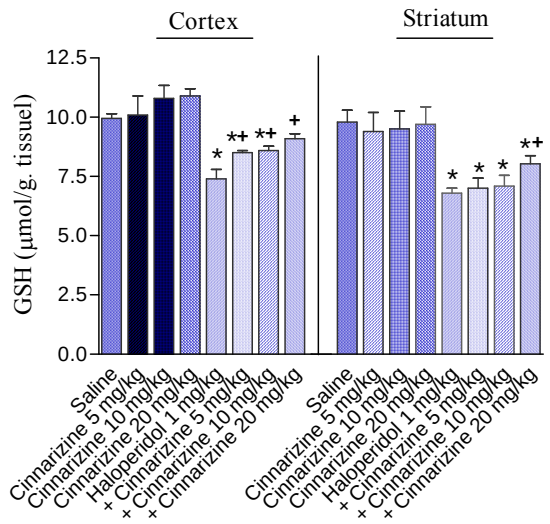
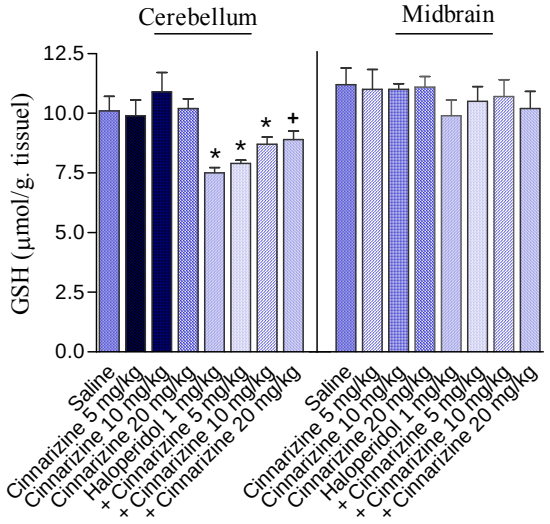


Fig. 5B



Figures 5A, B: Reduced glutathione (GSH) in cortex and striatum (A) and in cerebellum and midbrain (B) in mice treated with cinnarizine, haloperidol or cinnarizine + haloperidol for one month. *: P< 0.05 *vs* corresponding saline control value. +: P< 0.05 *vs*. haloperidol only-treated group

Nitric oxide

The level of nitric oxide in the cortex was reduced by 30.5 % after haloperidol treatment, compared with the saline-treated group (39.8 ± 2.1 *vs* 30.5 ± 2.6 µmol/g, $p<0.05$). Cinnarizine alone did not alter nitric oxide. In addition, no significant changes in the level of nitric oxide were observed in cinnarizine + haloperidol compared with the haloperidol only treatment group (Figure 6).

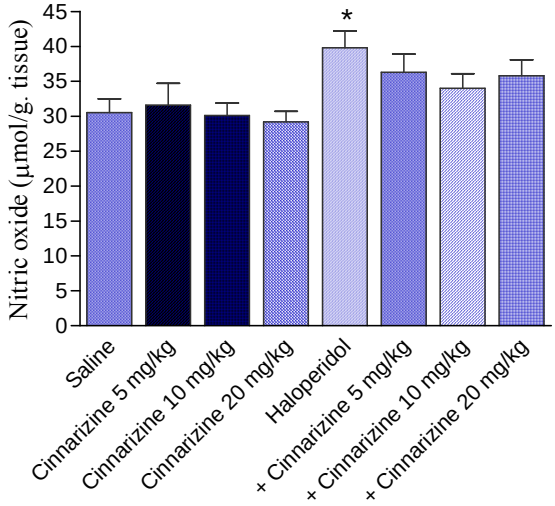


Figure 6: Nitric oxide in brain (cortex) of mice treated with cinnarizine, haloperidol or cinnarizine + haloperidol for one month. *: P< 0.05 vs corresponding saline control value

DISCUSSION

The present study examined the effect of the typical antipsychotic drug haloperidol and the calcium channel blocking agent cinnarizine on working memory and brain oxidative stress in mice. Both drugs are well known for their propensity to cause extrapyramidal symptoms in humans and in experimental animals, although for cinnarizine, high doses and old age are important predisposing factors (Thanvi and Treadwell, 2009). In light of the above, it seemed likely that greater inhibition of the dopamine D2 receptors by the combined treatment with both cinnarizine and haloperidol would result in profound memory impairment as well as markedly increased oxidative stress. The administration of haloperidol to mice impaired learning and memory. Both the acquisition and retention of infor-

mation were impaired by the drug. Mice spent more time to locate the submerged platform compared with their normal counterparts, which is in accordance with other published results (Abdel-Salam and Nada, 2011). Meanwhile, the administration of cinnarizine, improved the memory impairment observed in haloperidol-treated mice. In the present study, the dose of haloperidol used was shown to impair locomotor activity in other studies (Invernizzi et al., 2003) and a drug influence on motor activity thus cannot be ruled out. The water maze test, however, is primarily a test of cognition (spatial learning/memory) (Hamm et al., 1992) and the negative effects of haloperidol in the water maze task are likely to be memory-related (Terry et al., 2003). Studies indicated impairment of learning and memory by haloperidol. Haloperidol and several atypical neuroleptics e.g., clozapine, olanzapine, and risperidone impaired place navigation in the MWM when administered 30 min before testing (Skarsfeldt, 1996). Haloperidol caused impairment in memory retention observed by increased latency time to find the original location of platform in water-maze task (Barcelos et al., 2010). Haloperidol as well as the atypical antipsychotic clozapine impaired acquisition process and consolidation process, respectively, in step-through test. Both drugs impaired spatial learning function of mice in water maze task (Hou et al., 2006). The chronic administration of haloperidol significantly impaired learning performance in rats when compared to both vehicle controls and the atypical agents together with reduced choline acetyltransferase staining in several brain regions, including the cortex, caudate-putamen, and hippocampus (Terry et al., 2003). Both haloperidol and risperidone produced diminished motor activity and exploration, impaired working memory performances, and increased anxiety levels. These effects were more pronounced in haloperidol-treated animals (Karl et al., 2006). In man, haloperidol significantly attenuated error-related negativity, impaired learning of time intervals, and tended to cause more errors of commission, compared to placebo (Zirnheld et al., 2004**)**. The drug also worsened the immediate free and delayed free and cued recall deficits produced by Delta-9-tetrahydrocannabinol (D'Souza et al., 2008).

The dopaminergic system plays an important role in memory processes (Wisman et al., 2008; Costa et al., 2008; Schott et al., 2006; Bäckman et al., 2011). In humans, working memory capacity correlated positively with striatal (caudate) dopaminergic function and predicted dopamine synthesis capacity in the striatum, where subjects with low working memory capacity have low dopamine synthesis capacity in the striatum and *vice versa* (Cools et al., 2008). In support of this notion also is the finding that dopamine transmission is abnormal in the brains of patients with schizophrenia and working memory deficit is a core dysfunction in schizophrenia (Tanaka, 2006). In patients with Parkinson's disease, L-dopa administration significantly improved performance, restoring the prospective memory deficit, possibly through the replacement of dopamine levels in fronto-striatal pathways (Costa et al., 2008). In healthy volunteers, increased release of endogenous dopamine in the dorsomedial aspect of posterior putamen and in the anterior part of the caudate bilaterally was observed, during task performance (explicit motor memory task) (Badgaiyan et al., 2008). Chronic blockade of dopamine D2 receptors by antipsychotic drugs, down-regulates D1 receptors in the prefrontal cortex and produces severe impairments in working memory, which could be reversed by Dopamine D1 receptor stimulation (Castner et al., 2000). However, a greater contribution of D2- over D1-like receptors to both spatial working memory and object-location associative memory seems more likely (Von Huben et al., 2006). Drugs with dopaminergic agonist activity have been shown to improve memory function. Thus, piribedil displayed beneficial effects against aging-related memory impairments in two radial-maze experiments in mice (Marighetto et al., 2008) and pergolide facilitated spatial memory and improved brain oxidative

balance in experimental Parkinson's disease (Ciobica et al., 2012).

Meanwhile, there are only few reports that have explored the effects of cinnarizine on working memory in man. Parrott and Wesnes (1987) showed that cinnarizine significantly impaired psychomotor performance, information processing and feelings of alertness. In young healthy volunteers, oral cinnarizine was found to be less likely than hyoscine to produce unwanted decrements in performance (Golding et al., 1989). Cinnarizine (and scopolamine) also impaired memory error and four choice reaction time (Parrott et al., 1990). In the present study, cinnarizine failed to affect working memory in mice and even improved memory impairment due to haloperidol. Cinnarizine exhibits a dopamine D2 receptor blocking property and might therefore impair working memory. The drug, however, has been shown to exhibit a potential antipsychotic effect with an atypical profile (Dall'Igna et al., 2005). Cinnarizine also possesses other important pharmacological actions that could be of relevance to its effects observed in the present study. Cinnarizine can influence cognitive functions by activating sigma-1 receptors in the CNS. Sigma-1 agonists show powerful antiamnesic and neuroprotective effects in a large variety of animal models of cognitive dysfunction (Van Waarde et al., 2011). Cinnarizine **(**and its di-fluorinated derivative flunarizine) is a calcium channel antagonist and has been shown to exhibit antiinflammatory, antidepressant (Abdel-Salam, 2007) as well as neuroprotective effects (Belostotskaia et al., 2003). Cinnarizine (50 mg/kg, twice per day) prevented the hypoxic brain edema development, restored NAD+ dependent oxidation of a succinate substrate, normalized emotional-exploratory activity, and caused hyperlocomotion of the experimental animals, while not influencing a high level of activity of the blood antioxidant system (Belostotskaia et al., 2003). Cinnarizine (and flunarizine) at low concentrations (< 50 microM) inhibited the mitochondrial permeability transition induced either by Ca^{2+} alone or in the presence of tert-butylhydroperoxide. This was accompanied by the inhibition of NADPH oxidation and the restoration of the mitochondrial membrane potential decreased by a high concentration of Ca^{2+} (25 microM) (Elimadi et al., 1998). In other studies, flunarizine significantly facilitated memory retention (Petkov et al., 2000). Cinnarizine and flunarizine inhibited catecholamine uptake in storage vesicles (Terland and Flatmark, 1999), an effect which might have important implications on memory processing.

In the present work, the effect of both haloperidol and cinnarizine on brain oxidative stress in mice was studied. The administration of haloperidol resulted in increased MDA, nitric oxide and decreased GSH in several brain areas. The effect of haloperidol on MDA and GSH were mitigated by co-treatment with cinnarizine. This finding together with the improvement in working memory by cinnarizine administration, suggests that oxidative stress contributes to the haloperidol-induced learning and memory deficits in mice. Several studies indicated increased oxidative stress after the administration of haloperidol in rodents. Chronic treatment with haloperidol induced a significant decrease in the cellular glutathione content in selected areas of the brain (cerebellum, striatum and cortex) and in the liver (Vairetti et al., 1999). Haloperidol also induced a significant decrease in ATP and energy charge (Vairetti et al., 1999). In mice, haloperidol (1 mg/kg, i.p.) administered for 18 days, increased MDA, decreased GSH and increased nitric oxide in brain (Abdel-Salam et al., 2012). In rats, chronic haloperidol (2 mg/kg/day) administration increased brain lipid peroxidation and decreased both manganese-superoxide dismutase and copper-zinc superoxide dismutase (Pillai et al., 2007). Increased lipid peroxidation in rat striatum was observed after treatment with haloperidol (1.5 mg/kg/day) for 28 days (Martins et al., 2008). Evidence suggested the involvement of free radicals in the development of behavioral hypersensitivity by haloperidol (Daya et al., 2011). Studies also have

shown increased oxidative stress in brain in association with the deficits in memory function induced by ethanol (Gönenç et al., 2005), intracerebral β-amyloid protein injection (Um et al., 2009; Lu et al., 2009), glucocorticoids (Sato et al., 2010) as well as in the SAMP8 mice strain which exhibits memory impairment by 12 months of age (Farr et al., 2003). In these studies, the administration of antioxidants e.g., α-lipoic acid, *N*-acetylcysteine, silibinin or melatonin improved memory performance together with alleviation of oxidative stress, thereby suggesting a role for the latter in the cognitive decline seen in these models. Modulation of oxidative stress might be a mechanism that underlies the beneficial effect of cinnarizine on the haloperidol-induced memory impairment.

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