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RESEARCH ARTICLE

Invulnerability of bromelain against oxidative degeneration and cholinergic deficits imposed by dichlorvos in mice brains

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BACKGROUND: The present study elucidates the protective potential of bromelain against dichlorvos intoxication in mice brains. Dichlorvos induces the oxidative stress by disproportionating the balance between free radicals generation and their scavenging in neurons which leads to neuronal degeneration.
METHODS: In this study, mice were divided into four groups- group I (control), group II (dichlorvos treated), group III (bromelain treated) and group IV (exposed to both bromelain and dichlorvos both).
RESULTS: Dichlorvos treatment increased the levels of thiobarbituric acid reactive substances (TBARS) and protein carbonyl content (PCC) which indicate the increased oxidative stress. Meanwhile, brain endogenous antioxidants and cholinesterases level was decreased after dichlorvos exposure. Levels of TBARS and PCC decreased whereas cholinesterases level was recorded to be elevated after bromelain exposure.
CONCLUSION: Bromelain offered neuroprotection by decreasing oxidative stress and augmenting cholinesterases in mice brains. This study highlights the invulnerability of bromelain against oxidative and cholinergic deficits in mice brains.

Keywords oxidative stress, dichlorvos, bromelain, neuroprotection, neurotransmitter

Introduction

Dichlorvos is an organophosphate pesticide (OP) which is used in agriculture to protect plants, fruits and vegetables from insects (Chaudhary et al., 2014). It is highly toxic via inhalation, dermal absorption and ingestion (Gallo and Lawryk, 1991). Exposure to dichlorvos adversely affects almost all organs in the body but majorly affect is on the nervous system (Raheja and Gill, 2002; Cankayali et al., 2005). It is known to be a classical acetylcholinesterase (AChE) inhibitor (Assis et al., 2007). Dichlorvos can lead to generation of free radicals, inhibition of enzymatic activities and changes in the neurotransmitters (NTs) levels like dopamine, AChE and norepinephrine in the brain (Choudhary et al., 2002; Binukumar et al., 2010). Disproportion between the radical-generating and radical scavenging systems may cause an increase in free radical production or decrease in the antioxidant defense (Jones, 2006; Kangralkar et al., 2010). Treatment with dichlorvos increases the levels of malondialdehyde (MDA) and reactive oxygen species (ROS) and diminishes the activities of antioxidant enzymes (Eroglu et al., 2013). Dichlorvos is reported to cause the cholinergic toxicosis (Assis et al., 2007) and central apnea (Gaspari and Paydarfar, 2011) in the nervous system. It inhibits the AChE (Schulz et al., 1995; Hinz et al., 1996; Abdelsalam, 1999; Assis et al., 2012; Atanasov et al., 2013; Silva et al., 2013) and glutathione reductase (GR) activities in the brain which are biomarkers of neurotoxicity and oxidative stress (Pena-Llopis et al., 2003). Oxidative stress, NTs levels and antioxidant status are essential indices of dichlorvos induced toxicity in the brain which can be attenuated by natural antioxidants supplements. In the present study bromelain was attempted to ameliorate the toxic effects of dichlorvos in mice brains.

Bromelain is the proteolytic enzyme which is found in the pineapple plant (*Ananas comosus*). Bromelain is reported to have anti-inflammatory (Lotz-Winter, 1990; Maurer, 2001; Hale et al., 2005), anticarcinogenic (Maurer, 2001), immunomodulatory (Eckert et al., 1999; Hale et al., 2002; Hale, 2004), antidiarrheal, (Chandler et al. 1998), cardiovascular and circulatory improvement (Bhattacharyya, 2008) effects. It

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is also reported to have wound healing action (Roger and Orsini, 2006). Besides acting as an anti-inflammatory agent by increasing the permeability of the blood-brain barrier to nutrients, bromelain has been shown to have potential for future applications in the treatment of Alzheimer's disease patients (Lauer et al., 2001).

Material and methods

Chemicals and monitoring of animals

Dichlorvos, bromelain, acetylthiocholine iodide and butyrylthiocholine iodide were purchased from the Sigma Chemicals Company (St. Louis, MO, USA). 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and 2-thiobarbituric acid were purchased from Himedia (Chandigarh, India). Nitroblue tetrazolium (NBT) was purchased from SRL (Mumbai, India). All other chemicals used in present investigation were of analytical grade.

Healthy 6–8 weeks old, male Swiss mice (*Mus musculus*) were procured from C.C.S. Haryana Agriculture University, Hisar. Maintenance and treatment of animals was done in accordance with Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

Experimental design

The investigation was carried out in four groups of animals:
Groups Treatment
Group I Control (normal saline as a vehicle) -
Group II Dichlorvos (40mg/kg bodyweight (b.w.))
Group III Bromelain (70mg/kg b.w.)
Group IV Dichlorvos + Bromelain (40mg/kg b.w. + 70mg/kg b.w.)
Dichlorvos and bromelain were dissolved in water in separate vials. Dichlorvos was injected intraperitoneally and bromelain was given orally. The duration of exposure of different doses was 21 days.

Methods

After 21 days of duration of treatment control and treated mice were sacrificed by cervical dislocation. Brains were immediately removed and homogenized. Homogenization of tissue was done in phosphate buffer (pH 7.0). Homogenate was used for biochemical and molecular studies.

Oxidative stress and endogenous antioxidant assay
Thiobarbituric reactive substances (TBARS) and protein carbonyl content (PCC) were used as a biomarker of oxidative stress. TBARS levels were measured using the method of Ohkawa et al. (1979) method. PCC level will be measured using the method of Reznick and Packer (1994). Superoxide dismutase (SOD) activity was measured using the method of Dhindsa et al. (1981). SOD activity was measured by recording change in absorbance at 560 nm. The catalase (CAT) activity was determined using the method of Claiborne (1985). Reduced glutathione (GSH) level served as an index for determining the extent of lipid peroxidation. GSH level was determined using the method of Ellman (1959). Glutathione S-transferase (GST) was measured using the method of Habig et al. (1974). Glutathione peroxidase (GPx) activity was determined using the method of Mohandas et al. (1984). GPx inactivates hydrogen peroxide and provides protection against oxidative stress.

Brain cholinesterases
Thiocholine released from acetylcholine by the action of AChE, reacts with the DTNB, reducing it to a thiol entity which has absorption at 412 nm. Considering these principle activities of AChE and butyrylcholinesterase (BChE) were determined using the method of Ellman et al. (1961).

Results

TBARS

In mice brains dichlorvos increased TBARS level as compared to control and groups III ($p < 0.01$) and IV ($p < 0.01$). A significant decrease in TBARS level was found in group III compared to groups I and II ($p < 0.01$). Minimum level of TBARS was found in the mice that were treated with bromelain and dichlorvos both compared to groups I, II and III ($p < 0.01$) (Fig.1).

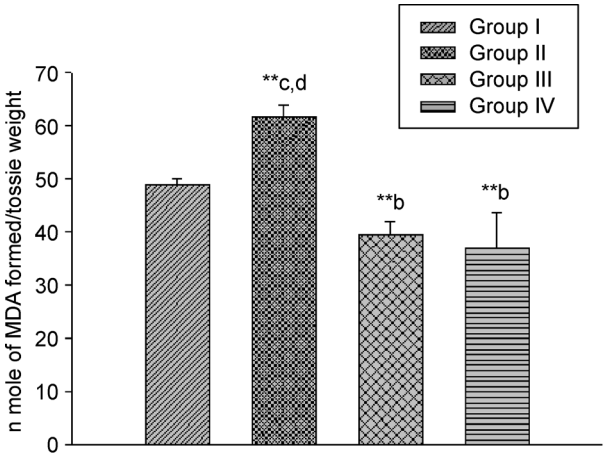

Figure 1 TBARS levels in mice brain of differently treated groups. Results are expressed as mean±S.E. ** = $p < 0.01$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

PCC

PCC was found to be significantly increased by the exposure of dichlorvos as compared to groups I, III and IV ($p < 0.001$).

After bromelain exposure PCC declined in brain of mice as compared to control and group II ($p < 0.001$) whereas a simultaneous increased was recorded as compared to group IV ($p < 0.01$). In group IV mice, PCC was found to be minimum and it significantly decreased from groups I ($p < 0.01$), II ($p < 0.001$) and III ($p < 0.01$) (Fig.2).

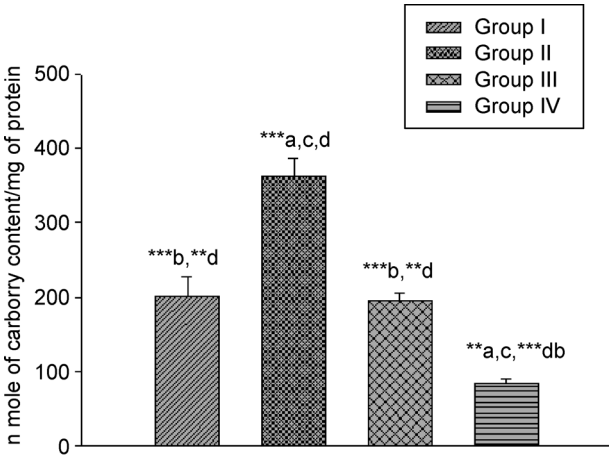

Figure 2 PCC in mice brain of differently treated groups. Results are expressed as mean±S.E. ** = $p < 0.01$ and *** = $p < 0.001$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

SOD

Enzymatic activity of SOD declined in dichlorvos treated mice compared to groups I ($p < 0.05$), III and IV ($p < 0.01$). Increased activity of SOD was found in group III compared to groups I and II whereas it declined compared with group IV. In group IV mice, SOD level was found to be maximum among all groups and it increased significantly when compared with group II ($p < 0.01$) (Fig.3).

CAT

Decreased CAT activity was found in brain of mice belonging to group II compared to groups I and III. Bromelain treatment enhanced the CAT activity compared to dichlorvos treated mice. However, enzymatic activity however declined in group III compared to group I. Activity of CAT decreased in group IV compared to all other groups (Fig.4).

GSH

Decreased GSH level was found in dichlorvos treated mice compared to groups I and III ($p < 0.001$). GSH activity was increased after bromelain administration compared to groups I, II ($p < 0.001$) and IV ($p < 0.001$). GSH level diminished in mice of group IV compared to groups I ($p < 0.001$), II and III ($p < 0.001$) (Fig. 5).

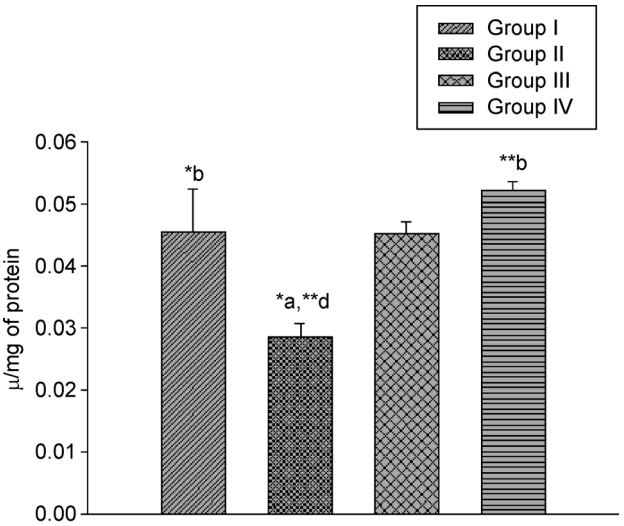

Figure 3 SOD levels in mice brain of differently treated groups. Results are expressed as mean±S.E. * = $p < 0.05$ and ** = $p < 0.01$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

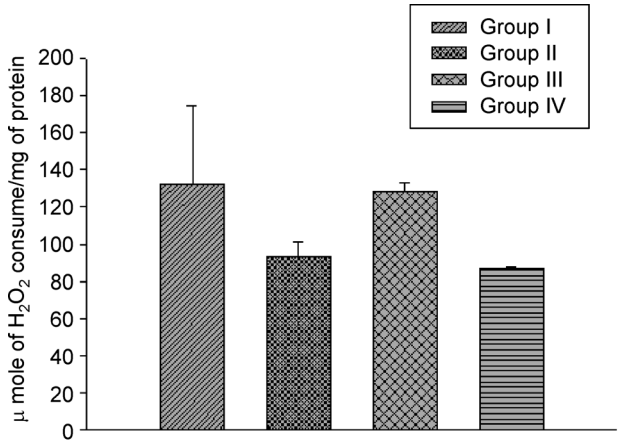

Figure 4 CAT levels in mice brain of differently treated groups. Results are expressed as mean±S.E.

GST

Level of GST decreased in brain of dichlorvos treated mice compared to groups I, III ($p < 0.001$) and IV ($p < 0.001$). A significant increase in GST activity was found in the brain of mice belonging to group III as compared to groups I, II ($p < 0.001$) and IV ($p < 0.001$). In group IV, a significant decline in GST activity was found compared to groups I and III ($p < 0.001$) however it increased compared to group II (Fig.6).

GPx

GPx activity decreased in brain of mice belonging to group II compared to control ($p < 0.01$) with simultaneous increase

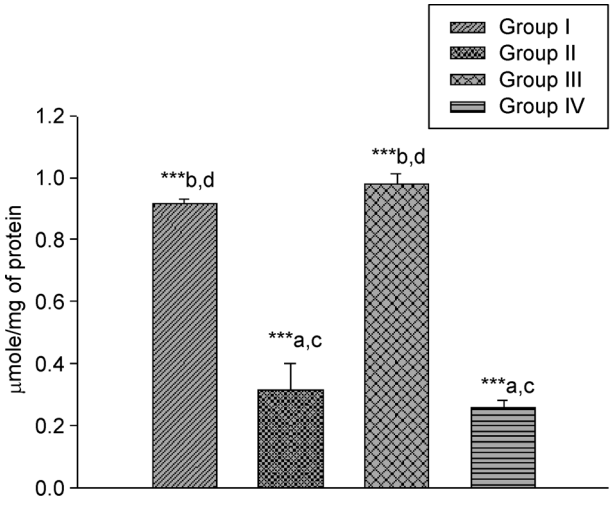

Figure 5 GSH activities in mice brain of differently treated groups. Results are expressed as mean±S.E. *** = $p < 0.001$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

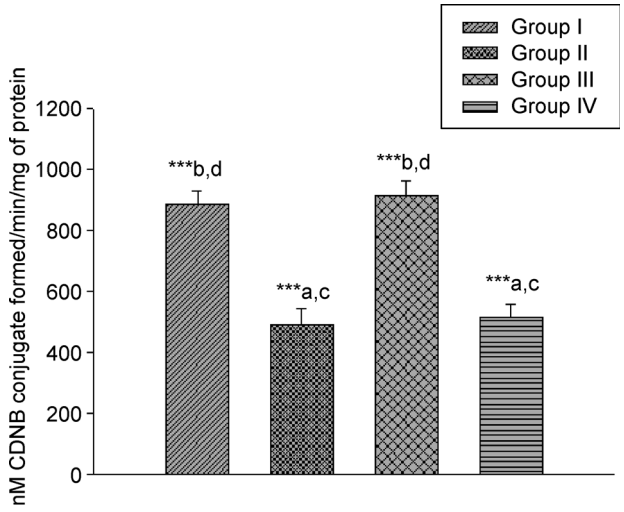

Figure 6 GST activities in mice brain of differently treated groups. Results are expressed as mean±S.E. *** = $p < 0.001$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

compared to group III. Level of GPx was decreased in group III mice compared to groups I ($p < 0.01$), II and III. An increase and decrease in GPx activity of group IV mice were obtained compared to groups III and I respectively (Fig.7).

AChE

Activity of AChE decreased in mice brain after the administration of dichlorvos compared to groups I, III ($p < 0.01$) and IV. A significant increase in AChE activity was found in the brains of group III mice compared to groups I, II ($p < 0.01$) and IV ($p < 0.01$). In group IV mice, a significant decrease in AChE activity was recorded compared to groups I and III ($p < 0.01$) however it slightly increased as compared to dichlorvos treated mice (Fig.8).

BChE

BChE activity decreased in dichlorvos treated mice compared to groups I, III and IV ($p < 0.05$). Increment in BChE activity was recorded in group III compared to group I and II. The enzymatic activity decreased in the bromelain treated mice as compared to group IV. Activity of BChE increased in group IV compared to groups I, II ($p < 0.05$) and III (Fig.9).

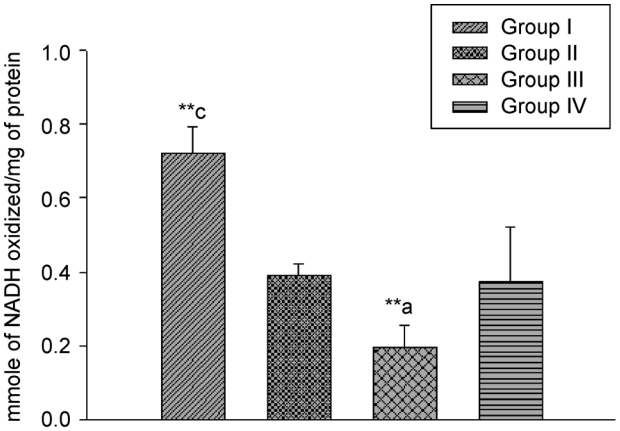

Figure 7 GPx levels in mice brain of differently treated groups. Results are expressed as mean±S.E. ** = $p < 0.01$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

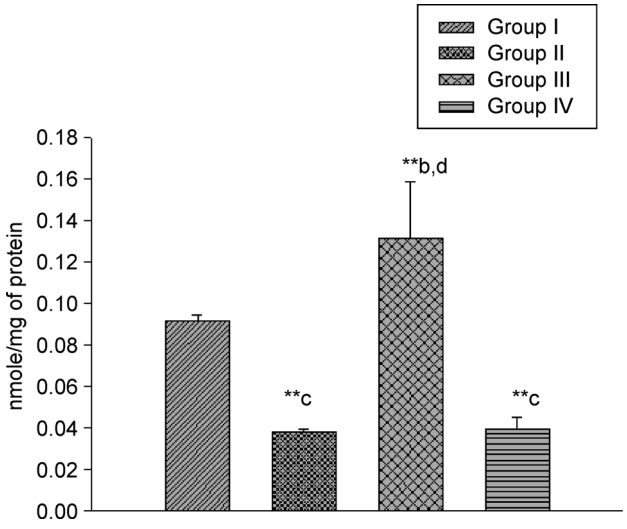

Figure 8 Activities of AChE in mice brain of differently treated groups. Results are expressed as mean±S.E. ** = $p < 0.01$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

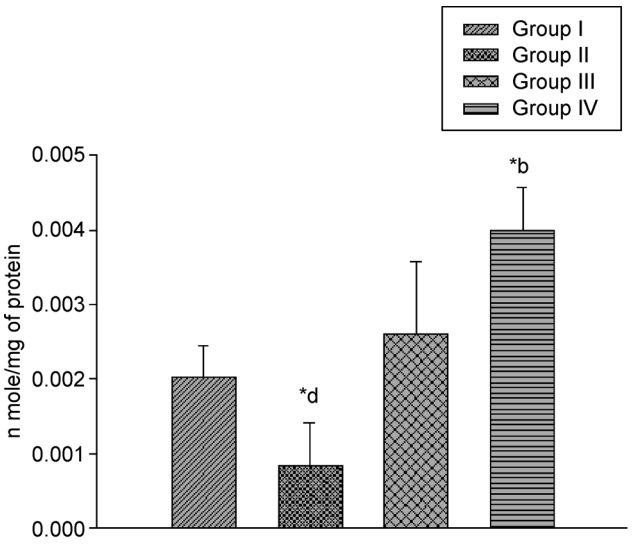

Figure 9 Activities of BChE in mice brain of differently treated groups. Results are expressed as mean±S.E. * = $p < 0.05$, a = compared to group I, b = compared to group II, c = compared to group III, d = compared to group IV.

Discussion

An imbalance in the antioxidant defense mechanism leads to neurological disturbance and neuronal cell death. The current study showed that oxidative stress increased and the levels of brain endogenous antioxidants and cholinesterases decreased after dichlorvos intoxication. When free radicals are generated excessively, the balance between formation and removal of reactive oxygen species is lost (Yoshikawa and Naito, 2002). This imbalance has been studied in current research by measuring the levels of TBARS and PCC which are two indices of oxidative stress. TBARS and PCC level were found to be increased after dichlorvos treatment which is in agreement with the findings by Yadav et al. (2012) and Kaur et al. (2007). It may be due to formation of hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and hydroperoxyl radicals (HOO^-) consequently leading to oxidative stress. Brain is known to have high concentration of iron which may be involved in the generation of hydroxyl radicals by iron catalysis. Hydroperoxyl radicals have role in lipid peroxidation (Birben et al., 2012). Production of peroxides and other free radicals in the normal redox state of cell can lead to toxic effects which may damage all the components of the cells. In the present study, bromelain exposure decreased the level of TBARS and PCC content. Habashi et al (2012) reported that bromelain significantly reduced the production of nitric oxide in the rat primary microglia. TBARS and PCC level were decreased by Concomitant exposure of dichlorvos and bromelain since bromelain has property to diminish the oxidative stress through minimizing the ROS production and halting the protein oxidation.

In the current study, activities of CAT, GST, GPx, SOD and GSH decreased in dichlorvos treated mice which are supported with the findings of Sharma and Singh (2012). Glutathione level is regarded as an index of oxidative stress. GST plays a crucial role in detoxifying ROS in conjugation of GSH through sulfhydryl groups and making more xenobiotics (Xing et al., 2012). Furthermore, the decrease in CAT and GST may be mechanism for the detoxification of dichlorvos in the brain. Bromelain increases GPx, SOD and GSH level. No much difference was recorded in activities of GST and CAT in bromelain treated mice compared to control. Simultaneous administration of bromelain and dichlorvos in mice resulted in decreased level of GSH, GPx and CAT compared to dichlorvos treated mice. Meanwhile, the concomitant exposure of bromelain and dichlorvos enhanced the activities of SOD, GST, AChE and BChE. It indicates that bromelain exerts its protective action via altering the molecular cascades involving SOD, GST, AChE and BChE. However, GSH, GPx and CAT are unaffected by the same.

Dichlorvos is effective in pest control (ATSDR, 1997; U.S. EPA, 2006) and produces toxicity primarily through AChE inhibition (Carod-Artal, 1999; Sarin and Gill, 1999; Yadav et al., 2012). In the present study, inhibition of AChE and BChE activity was observed after the dichlorvos administration. Previous study by Celik et al. (2009) reported that activity of AChE was inhibited after the dichlorvos exposure which may result due the accumulation of Ach in synaptic cleft leading to overstimulation of post synaptic cells and cholinergic manifestation (Savolain, 2001; Sharma et al., 2005; Shenouda et al., 2009). Treatment with bromelain increased the level of AChE and BChE. Exposure of bromelain along the dichlorvos increased the AChE and BChE level which imply that bromelain has property to boost up the cholinergic system. Moreover the levels of extracellular ACh in the brain are controlled by the activity of BChE in the absence of AChE (Hartmann et al., 2008). Low level of AChE in the brain contributes to induction of Alzheimer's disease. Bromelain increases the permeability of the blood-brain barrier to nutrients, therefore it has shown certain possibilities for therapeutic application to Alzheimer's disease (Lauer et al., 2001).

In conclusion, it can be safely inferred that dichlorvos manifests its toxic actions by declining the brain endogenous antioxidants and cholinesterase and elevating the degree of oxidative stress. Bromelain has neuroprotective potential to eradicate the toxicity imposed by dichlorvos.

Abbreviations

Acetylcholinesterase (AChE)
Butyrylcholinesterase (BChE)
Catalase (CAT)
Glutathione peroxidase (GPx)
Reduced glutathione (GSH)
Glutathione reductase (GR)

Glutathione-S-transferase (GST)
Malondialdehyde (MDA)
Neurotransmitters (NTs)
Protein carbonyl content (PCC)
Reactive oxygen species (ROS)
Superoxide dismutase (SOD)
Thiobarbituric acid reactive substances (TBARS)

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Compliance with ethics guidelines

Bharti Chaudhary, Sonam Agarwal and Renu Bist declare that they have no conflict of interest.

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