Lacidipine Attenuates Symptoms of Nicotine-Withdrawal in Mice

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Research Article

Keywords: Lacidipine, Nicotine, Bay-K8644, Memory, Depression, Anxiety

Posted Date: June 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-635075/v1

Version of Record: A version of this preprint was published at Neurotoxicity Research on October 6th, 2021. See the published version at https://doi.org/10.1007/s12640-021-00421-2.

Abstract

Nicotine-withdrawal after daily exposure manifests somatic and affective symptom including a range of cognitive deficits. Earlier studies suggested participation of L-type calcium channels (LTCCs) in development of nicotine dependence and expression of withdrawal signs. An upsurge in Ca^{2+}-induced oxidative stress in brain underlies the biochemical events and behavioral signs of nicotine-withdrawal. The present study is aimed to explore the effects of lacidipine (LTCC antagonist) against nicotine-withdrawal. Swiss albino mice were administered (−)-nicotine hydrogen tartrate (3.35 mg/kg, *t.i.d.*) from day 1 to 7 and alongside lacidipine (0.3, 1 and 3 mg/kg, *i.p.*) given from day 1 to 14. Somatic withdrawal signs were noted 48 h after last dose of nicotine. Bay-K8644 (LTCC agonist) was administered in mice subjected to nicotine-withdrawal and lacidipine (3 mg/kg) treatments. Behavioral tests of memory, anxiety, and depression were conducted on day 13 and 14 to assess the effects of lacidipine on affective symptoms of nicotine-withdrawal. Biomarkers of oxido-nitrosative were quantified in the whole brain. Nicotine-withdrawal significantly enhanced somatic signs and symptoms of anxiety, depression, and memory impairment in mice. Lacidipine (1 and 3 mg/kg) attenuated nicotine-withdrawal induced somatic symptoms and also ameliorated behavioral abnormalities. Nicotine-withdrawal triggered an upsurge in brain lipid peroxidation, total nitrite content, and decline in antioxidants and these effects were attenuated by lacidipine. Bay-K8644 significantly abolished improvement in somatic and affective symptoms, and antioxidant effects by lacidipine in mice subjected to nicotine-withdrawal. Lacidipine mitigated nicotine-withdrawal triggered somatic and affective symptoms owing to decrease in brain oxido-nitrosative stress.

Introduction

Nicotine is identified as the major factor contributing to tobacco dependence. Multiple attempts to quit tobacco consumption with high relapse rates are linked with nicotine dependence (Fowler and Kenny 2014). Nicotine is a psychostimulant alkaloid present in tobacco that manifests hedonic and non-hedonic bio-effects including organ toxicity, adverse reactions, and carcinogenic potential (McLaughlin et al. 2015). Some hedonic effects of nicotine include a feeling of pleasure, anorexia, and cognitive improvements (Volkow et al. 2019; Isomura et al. 2014; De Biasi and Dani, 2011; Barrett et al. 2004). Small doses of nicotine or tobacco consumption are often associated with relief in stress (Holliday and Gould 2016), pain (Ditre et al. 2016; Ditre et al. 2011), and symptoms of anxiety (Morissette et al. 2007; File et al. 1998). However, with repeated exposure, tolerance develops to some of the behavioral effects of nicotine (Benowitz 2009; Mishra et al. 2015). It is observed that sudden termination of exposure to nicotine or administration of nicotinic cholinergic receptors (nAChR) antagonist in animals chronically exposed to nicotine precipitates behavioral symptoms akin to opiate withdrawal (Watkins et al. 2000). Nicotine abstinence manifests withdrawal symptoms (somatic, affective, and cognitive) such as tremors, decreased heart rate, discomfort in the gastrointestinal tract, appetite exacerbation, hyperalgesia, anhedonia, dysphoria, irritability, and a range of behavioral changes (e.g., anxiety, loss of memory, depression) (Foll and Goldberg 2009; Cohen and George 2013).

Nicotine triggers the influx of Na^+ and Ca^{2+} ions *via* L-type voltage-dependent Ca^{2+} channels (LTCCs) and postsynaptic nAChRs (Katsura et al. 2002). This cationic influx depolarizes the dopaminergic neurons at the ventral tegmentum (VTA), thereby eliciting dopamine (DA) output at the nucleus accumbens (NAc) and amygdala regions of the brain. Experimental data also indicates that nicotine abstinence suppresses dopamine release at the ventral striatum (Yasuno et al. 2007). Nicotine-triggered glutamatergic signaling further potentiates the DA release (Alasmari et al. 2016; D'Souza and Markou 2013; Watkins et al. 2000). DA at NAc mediates the reward and hedonic effects of nicotine that are critical for nicotine addiction (Benowitz 2009; McLaughlin et al. 2015). Desensitization and upregulation of nAChRs with a relative reduction in GABA (inhibitory neurotransmitter) release at NAc is associated with tolerance and dependence on nicotine (Foll and Goldberg 2009; Benowitz 2009). Nicotine modulates many more neurotransmitters such as acetylcholine, serotonin, GABA, and opioid-peptides that are associated with withdrawal symptoms (D'Souza and Markou 2011). However, nicotine-induced increase in expression of LTCCs suggests the involvement of Ca^{2+} in nicotine sensitization (Bernardi et al. 2014). In previous studies, two different L-type Ca^{2+} channel subtypes have been associated with the origin of early-exposure nicotine dependence and appearance of nicotine withdrawal symptoms (Liu et al. 2017; Bernardi et al. 2014). A significant increase in intracellular Ca^{2+} is the basis of free radical toxicity, inflammation, and brain atrophy in nicotine addicted persons (Malinska et al. 2019). Auto-oxidation of neurotransmitters also potentiates the Ca^{2+} triggered pathogenesis, such as mitochondrial dysfunction, reactive oxygen species (ROS), lipid peroxidation, and cell death pathways (Dong et al. 2009). Evidence from previous studies suggest that restrain over oxidative and inflammatory cascade may attenuate the nicotine dependence and withdrawal symptoms (Hanna 2006). Furthermore, it has been noted that L-type Ca^{2+} blockers (*e.g.*, nifedipine) (Bernardi et al., 2014) not only attenuate the redox-imbalance but also modulate neurotransmitter release that regresses the involvement of LTCCs in the development of nicotine-withdrawal (Jackson and Damaj 2009; Biala et al. 2014).

Lacidipine (LCD) is a 1,4-dihydropyridine type L-type calcium channel antagonist that possesses an extended duration of action (Lee and Bryson 1994). It dilates peripheral and coronary blood vessels by acting directly on vascular smooth muscles that renders its use as an antihypertensive agent alone or in combination with other antihypertensive agents and in many cardiovascular disorders (McCormack and Wagstaff, 2003). Lacidipine exhibits antioxidant activity equivalent to vitamin E and antioxidant effects of lacidipine are significantly higher than other dihydropyridine type calcium channel antagonists (Cominacini et al. 2003). It also suppresses inflammatory chemokines, cytokines (*e.g.*, tumor necrosis factor-*α*, interleukins), and release of adhesion molecules (*e.g.*, selectins, integrins), which might alleviate cardiovascular abnormalities such as atherosclerotic lesion formation (Anacak et al. 2010). The antioxidative efficacy of lacidipine is attributed primarily to direct free radical scavenging activity (Godfraind and Salomone 2015) and inhibition of peroxidative changes by proton (H^+) transfer (Garzotti 2003). Lacidipine also imparts a protective effect on hepatic (Kamal 2003), renal (Micheli et al. 1991), and osseous organ systems (Halici et al. 2008). Lacidipine can easily permeate the blood-brain barrier owing to its highly lipophilic structure (McCormack and Wagstaff 2003; Bellosta and Bernini 2000; van Zwieten 1998), have a high membrane partition coefficient (Herbette et al. 1993), and thereby attains

significant concentration in the brain when administered orally or systemically (Lee and Bryson 1994). In our previous studies, lacidipine (1 and 3 mg/kg, *i.p.*) was able to reduce the symptoms of depression and anxiety in different animal models (Khurana and Bansal 2019a; Khurana and Bansal 2019b). The existing literature indicates that treatment with Ca^{2+} antagonists may alleviate the nicotine dependence and withdrawal symptoms (Biala et al. 2014; Katsura et al. 2002). We hypothesize that lacidipine may mitigate nicotine-withdrawal induced somatic and affective symptoms including memory deficits in mice owing to its potent antioxidant and Ca^{2+} channel blocking activities.

Materials And Methods

Experimental animals

Forty-eight Swiss albino mice (24 male and 24 female adults) of weight range 20-25 g were received from the CRI, Kasauli and LUVAS, Hisar after approval of the protocol no. ASCB/IAEC/08/15/108 (dated: 28-11-2015) by the Institutional Animal Ethics Committee (IAEC) at A.S.B.A.S.J.S.M. College of Pharmacy, Ropar, India. Animals were reserved in isolation till confirmation of their health status and then relocated to the housing section. Three mice of the same sex were kept in a single polyacrylic cage (30 × 23 × 14 cm^3) under typical laboratory situations with controlled humidity (40 ± 10%), temperature (23 ± 2°C), and light-dark cycle (12 h each). Rodents were provided two weeks for customization to the housing/laboratory environment prior to the experiments. Guiding principles of “The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)” GOI were pursued for the entire course of animal experiments and care of animals. Unrestricted access to typical rodent pellet diet (Ashirwad Industries, Mohali) and purified water was provided to the mice. Caretakers were blinded to treatments that the animals received. All the experiments and procedures were conducted within 12 h cycle (0800 to 2000 h).

Drug treatments

(−)-Nicotine hydrogen tartrate [(−)-1-Methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt] (Sigma-Aldrich, Bengaluru, India) was prepared freshly (dose 3.35 mg/kg) (Biala et al. 2014) using sterile 0.9% normal saline (pH 7.3, dose volume of 2 ml/kg). (−)-nicotine hydrogen tartrate (Nic) was administered subcutaneously (*s.c.*) from day 1 to 7 thrice a day (*t.i.d.*) at 5 h intervals (0900, 1400, and 1900 h) (Matta et al. 2007). The intraperitoneal (*i.p.*) doses of lacidipine (LCD), sertraline, and Bay-K8644 were constituted using a 0.2% dimethyl sulfoxide (DMSO) vehicle. Lacidipine (TCI Chemicals, Chennai, India) at doses 0.3, 1, and 3 mg/kg (*i.p.*) was injected at a dose-volume of 3 ml/kg once daily for 14 consecutive days initiating from day 1 (Khurana and Bansal 2019a; Bellosta et al. 2001). Sertraline (Zee Laboratories, Ponta Sahib, India), a selective serotonin reuptake inhibitor (SSRI), was taken as a standard drug in the current protocol and was administered (40 mg/kg, *i.p.*) in mice at dose-volume 4 ml/kg for 14 days once daily (Noori et al. 2014). LCD and sertraline were injected 60 min before Nic administration. Bay-K8644 (Sigma-Aldrich, Bengaluru, India) is an LTCC agonist and was given at a dose 0.5 mg/kg (dose-volume 2 ml/kg, *i.p.*) 120 min before behavioral tests on day 13 and 14 (Khurana and Bansal 2019b; Jackson and

Damaj 2009). The whole dose-volume was reserved ≤ 10 ml/kg body weight (*b.w.*) per day in each mouse.

Experimental protocol

Male and female mice were randomly divided into 8 different groups (n = 6) (Table 1) in a single-blind pattern by adopting a random distribution scheme with an equal number of male (n = 3) and female (n = 3) mice in each group (Miller et al. 2017): (i) Vehicle control group was administered 0.2% DMSO (*i.p.*) from day 1 to 14 and normal saline (*s.c.*) from day 1 to 7; (ii) LCD(3) group was administered lacidipine (3 mg/kg) from day 1 to 14 to observe the *per se* effects of the lacidipine on behavioral symptoms; (iii) Nic group served as nicotine-withdrawal control group that received (−)-nicotine hydrogen tartrate (3.35 mg/kg, *t.i.d.*) from day 1 to 7 only and drug vehicle from day 1 to 14; (iv) LCD(0.3)+Nic group was administered lacidipine (0.3 mg/kg) from day 1 to 14 and Nic (3.35 mg/kg, *t.i.d.*) from day 1 to 7; (v) LCD(1)+Nic group was administered lacidipine (1 mg/kg) from day 1 to 14 and Nic (3.35 mg/kg, *t.i.d.*) from day 1 to 7; (vi) LCD(3)+Nic group was administered lacidipine (3 mg/kg) from day 1 to 14 and Nic (3.35 mg/kg, *t.i.d.*) from day 1 to 7; (vii) Sertraline+Nic group was administered sertraline (40 mg/kg) from day 1 to 14 and Nic (3.35 mg/kg, *t.i.d.*) from day 1 to 7; (viii) Bay-K8644+LCD(3)+Nic group was administered Bay-K8644 (0.5 mg/kg) on day 13 and 14 before behavioral tests, lacidipine (3 mg/kg, 14 days) and (−)-nicotine hydrogen tartrate (3.35 mg/kg, *t.i.d.*, 7 days).

Nicotine-withdrawal symptoms were induced in mice using protocol given by Aggarwal and Bansal (2018). Mice were exposed to (−)-nicotine hydrogen tartrate (3.35 mg/kg, *s.c.*, *t.i.d.*) for seven days to develop nicotine dependence and thereafter, mice were refrained from further nicotine exposure to trigger symptoms of nicotine-withdrawal (Biala et al. 2014; Matta et al. 2007). After 48 h (*i.e.,* day 9) of the last dose of (−)-nicotine hydrogen tartrate, animals in groups (i) – (vii) were observed for 30 min to assign withdrawal signs scores (somatic withdrawal signs) *i.e.,* head shake, leg licking, rearing, grooming, jumping, and genital licking in a clean home cage (Isola et al. 1999; Biala et al. 2014). Each sign was assigned scores of one. Behavioral tests were conducted 60 min after drug treatments to assess the affective symptoms *viz.* memory, anxiety-like, and depressive symptoms in mice. All the groups were subjected to elevated plus maze (EPM) test, mirror chamber test, and tail suspension test (TST) on days 13 and 14. After behavioral tests, mice were euthanized by using the cervical dislocation method, and biochemical parameters of oxido-nitrosative stress were determined in the whole brain homogenate (Table 1).

Table 1. Experimental protocol to assess effects of lacidipine (LCD, Ca^{2+} antagonist) on nicotine-withdrawal signs and symptoms noted in mice that were given continuous exposure of (−)-nicotine hydrogen tartrate (Nic) at dose 3.35 mg/kg (*s.c.*, *t.i.d.*) for 7 days.

	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group (*n* = 6)														
(i) Vehicle control	0.2% DMSO + Normal saline							0.2% DMSO						
(ii) LCD(3)	LCD (3 mg/kg)													
(iii) Nic	Nic + DMSO							0.2% DMSO						
(iv) LCD(0.3) + Nic	LCD (0.3 mg//kg) + Nic							LCD (0.3 mg//kg)						
(v) LCD(1) + Nic	LCD (1 mg//kg) + Nic							LCD (1 mg//kg)						
(vi) LCD(3) + Nic	LCD (3 mg//kg) + Nic							LCD (3 mg//kg)						
(vii) Sertraline + Nic	Sertraline (40 mg/kg) + Nic							Sertraline (40 mg/kg)						
(viii) Bay-K8644 + LCD(3) + Nic	LCD (3 mg//kg) + Nic							LCD (3 mg//kg)					LCD (3 mg//kg) + Bay-K8644 (0.5 mg/kg)	

Vehicle control group was given drug vehicles and LCD(3) was administered LCD (3 mg/kg) for 14 days. Nic group was continuously exposed to nicotine for 7 days and thereafter, Nic administration was discontinued to trigger somatic and affective withdrawal signs. LCD (0.3, 1 and 3 mg/kg, *i.p.*) or sertraline (40 mg/kg, *i.p.*) were given for 14 days alongside Nic for 7 days in separate groups (iv – vii). In groups (i) – (vii), all the drugs were administered from day 1 onwards and somatic withdrawal signs were assessed on day 9. In group (viii), L-type Ca^{2+} agonist, Bay-K8644 (0.5 mg/kg), was injected before behavioral studies in mice that received LCD (3 mg/kg, 14 days) and Nic (dose 3.35 mg/kg, *t.i.d.* for 7 days) from day 1. All the groups were subjected to elevated plus maze (EPM) test on day 13 and 14, mirror chamber test on day 13, and tail suspension test (TST) on day 14.

Elevated plus maze test

Elevated plus maze test is widely used to assess spatial learning and memory in rodents across the globe. The standard protocol of EPM test was followed in the present study to assess memory abilities in mice (Bansal and Parle 2011). The EPM apparatus (wooden) consisted of two exposed sections (16 × 5 cm) and two enclosed sections (16 × 5 × 12 cm) contrary to each other hooked *via* an intermedial dais (5 × 5 cm), positioned at 40 cm height. Individual animals were placed at the edge of an exposed section facing opposite from the intermedial dais. Time (s) required by mouse to relocate from the exposed section into either of the enclosed sections with all its 4 paws was noted as transfer latency (TL). Mice not entering into either of the enclosed section within 90 s were gently guided into nearest enclosed section, and 90 s of TL was allotted. Each mouse was kept in the maze for an additional 10 s for exploration and thereafter transferred to their respective cages. During the acquisition trial (day 13) TL was observed (training session) for each moue. After 24 h of the acquisition trial, mice were evaluated for

retention of this learned task (memory), and TL was noted (day 14). A significant decline in TL value during the retrieval trials indicated improvement in spatial memory (Itoh et al. 1990).

Mirror chamber test

For evaluation of anxiety-like symptoms, an approach-avoidance behavior of mice in response to the mirror was utilized by employing a mirror chamber apparatus. Its design consisted of an outer large compartment made of plywood (40 cm length × 40 cm breadth × 30.5 cm height) and an inner mirror-compartment (35 cm length × 35 cm breadth × 30 cm height) positioned at the center of the large compartment that allows a 5 cm passage between walls of both compartments. Another mirror (6^{th}) was secured on the outer compartment wall in front of the exposed portion of the inner mirror-compartment. Individual mouse was positioned softly at a place in the passage at backside of mirror-compartment. Animal was permitted free access to all the parts if the apparatus including mirror-compartment for 5 min. Subsequently, (a) latency to enter the mirror chamber (s), (b) the number of entries, and (c) total time spent (s) in the mirror chamber were manually recorded (Toubas et al. 1990).

Tail suspension test

The method given by Steru et al. (1985) was implemented to measure passive motionless period of each animal that correlates to depression-like signs. Mice subjected to the short duration of inescapable stress show immobility which is a reliable index of depressive states. Individual animal was fastened (adhesive tape ~1 cm) by tip of the tail at an elevation of 58 cm above the table-top. Duration of static or motionless dangling of animal was measured in s for a 6 min period. A decrease in static period denotes antidepressant effect. The results of TST were stated as the duration of immobility (s).

Preparation of whole-brain homogenate

The mice were euthanized (n = 6) by cervical dislocation method. Instantly complete brain was harvested and bathed with freezing sterile isotonic normal saline and weight (g) noted. The brain was homogenized using tissue homogenizer (Remi Motors, Remi Electrotechnik, Vasai, India) in ice-cold 50 mM sodium-phosphate phosphate buffer (pH 7.40) at temperature 4°C to prepare 10% w/v brain homogenate. Subsequently, a clear supernatant was obtained by centrifuging (CPR-30 Remi Compufuge, Vasai, India) whole brain homogenate for 15 min at 4°C at 12,000 × g force. The clear supernatant was separated for evaluation of biochemical parameters.

Estimation of lipid peroxidation in the brain

Thiobarbituric acid reactive substances (TBARS) reflect lipid peroxidation and relates with malondialdehyde (MDA) production (Ohkawa et al. 1979). The analyze mixture (final volume 4 ml) comprised 0.10 ml test sample (homogenate), 1.50 ml thiobarbituric acid (TBA, 0.8%), 1.50 ml glacial acetic acid (20%, pH 3.50), 0.20 ml sodium dodecyl sulphate (SDS, 8.10%), and 0.70 ml purified water. The analyze mixture in test-tubes was mixed vigorously, boiled on a water bath for 1 h (temperature

95°C), and cooled under flowing tap water. *n*-butanol and pyridine (15:1) solution was added (5 ml) and centrifuged for 10 min at 4,000 × *g* force. The optical density (O.D.) of MDA-TBA_2 adducts (pink color) in the superior (*n*-butanol phase) 2 ml organic layer was noted at λ_{max} = 532 nm by means of a double beam UV-spectrophotometer (Shimadzu UV-1700, Pharmaspec). TBARS (nanomole/mg protein) quantified using $\varepsilon = 1.56\times10^5$ /M/cm.

Estimation of reduced glutathione (GSH) content

Test samples containing 1.0 ml of supernatant were precipitated using equivalent amount of 4% sulphosalicylic acid. The analyze mixture was cold-digested (4°C temperature) for 1 h and then centrifuged (4°C temperature) for 15 min at 2000 × *g* force. Supernatant was collected (0.10 ml) and mixed with 2.70 ml Na^+-K^+ PO_4^{3-} buffer (0.31 M, pH 8.1) and 0.20 ml 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB: 0.11 mM, pH 8.1). Standard curve of reduced GSH prepared (concentrations of GSH 0.2, 0.4, 0.6, 0.8, and 1 mM). The O.D. standard and tests were noted spectrophotometrically at λ_{max} = 412 nm and the amount of reduced GSH (micromole GSH/mg protein) quantified (Ellman 1959).

Determination of superoxide dismutase (SOD) activity

The activity of superoxide dismutase (SOD) was estimated by following the method of Winterbourn et al. (1975). Briefly, the test-tubes (final volume 3 ml) containing 0.05 ml supernatant of homogenized whole brain, 0.05 ml riboflavin (0.12 mM), 0.10 ml nitroblue tetrazolium (NBT, 1.50 mM), 0.20 ml of 0.10 M ethylenediaminetetraacetic acid (EDTA, 0.30 mM sodium cyanide NaCN), and Na^+/K^+ PO_4^{3-} buffer (67.0 mM, pH 7.81) *q.s.* were illuminated for 13 ± 3 min underneath 100 W fluorescent-tube (Bajaj®) and optical density variability (λ_{max} = 560 nm) noted. The SOD in the sample impedes the reduction of NBT by O_2 and formazan synthesis. SOD activity (μM NBT reduced per min per mg protein) quantified using ε (formazan) = 15,000 /M/cm.

Determination of catalase (CAT) activity

The analyze mixture (3.0 ml) contained 0.05 ml test sample (supernatant), 1.10 ml hydrogen peroxide (H_2O_2 0.02 M) in Na^+/K^+ PO_4^{3-} buffer (pH 7.80, 0.05 M), and 1.85 ml of 0.05 M Na^+/K^+ PO_4^{3-} buffer (pH 7.0). Change in O.D. noted spectrophotometrically at λ_{max} = 240 nm for 3 min at 30 s intermissions. Catalase (micromole H_2O_2 decomposed/min/mg protein) activity was quantified by means of ε = 43.60 /M/cm (Claiborne 1985).

Estimation of total nitrite content in the brain

The method given by Sastry et al. (2002) was adopted for determination of total nitrites. Briefly, the test-tubes containing 0.10 ml test samples (supernatant) or standard, alloy of Copper-Cadmium (150 mg), and 0.40 ml carbonic acid (H_2CO_3) buffer (pH 9.0) were subjected to incubation (1 h at ambient temperature). 0.10 ml NaOH (0.35 M) and 0.40 ml $ZnSO_4$ solution (120.0 mM) were added and analyze

mixture centrifuged at 4000 × *g* for 10 min and supernatant separated. Griess reagent (0.05 ml) was mixed with 0.1 ml of supernatant. After incubation of 30 min at room temperature in the dark, O.D. at λ_{max} = 548 nm was determined spectrophotometrically. A standard curve of $NaNO_2$ (concentration range 0.01-0.1 mM) was plotted, total nitrite content compared, and stated as μmole per mg of brain protein.

Estimation of total proteins in the brain of mice

The total protein content (mg/ml) was quantified using a typical curve of bovine serum albumin (BSA) with concentration range 0.2-2.4 mg/ml. The test blend was arranged using 0.25 ml test sample containing supernatant, Lowry's reagent (5.0 ml), and Na^+/K^+ PO_4^{3-} buffer (1.0 ml). After incubation (15 min, room temperature), Folin-Ciocalteu reagent (0.50 ml of 1.0 N) was added, mixed, and again incubated (30 min, room temperature). O.D. was determined spectrophotometrically at λ_{max} = 650 nm (Lowry et al. 1951).

Statistical analysis

Data were analyzed by an experienced experimenter blinded to diverse treatments received by different groups of mice. The data were analyzed by one-way ANOVA followed by Tukey's honest significant difference (HSD) *post-hoc* test or two-tailed paired t-test using software GraphPad Prism 5.0 (GraphPad Software Inc., California, USA). Two-way ANOVA and Bonferroni *post-hoc* test was used to evaluate the influence of sex on the outcome of different parameters studied in experiments. All results were expressed as mean ± standard error of mean (S.E.M.) and $p < 0.05$ was deemed to be statistically significant.

Results

Lacidipine decreased symptoms of nicotine-withdrawal in mice

Somatic manifestations post nicotine-withdrawal were noted in Groups I – VII on day 9, after a sudden withdrawal from sustained nicotine exposure for 7 days. Results showed that withdrawal symptoms manifested in mice in response to cessation of exposure to nicotine. Nicotine-withdrawal triggered a significant ($p < 0.001$) increase in somatic withdrawal signs such as rearing [$F_{(6, 35)} = 48.40$, $p < 0.001$], grooming [$F_{(6, 35)} = 86.01$, $p < 0.001$], jumping [$F_{(6, 35)} = 41.50$, $p < 0.001$], head shaking [$F_{(6, 35)} = 86.13$, $p < 0.001$], leg licking [$F_{(6, 35)} = 59.77$, $p < 0.001$], and genital licking [$F_{(6, 35)} = 38.42$, $p < 0.001$] in mice relative to vehicle given controls. Lacidipine (1 and 3 mg/kg) treatment significantly decreased the nicotine-withdrawal induced somatic symptoms such as rearing ($p < 0.05$; $p < 0.001$), grooming ($p < 0.05$; $p < 0.001$), jumping ($p < 0.05$; $p < 0.001$), head shaking ($p < 0.01$; $p < 0.001$), leg licking ($p < 0.05$; $p < 0.01$), and genital licking ($p < 0.05$; $p < 0.01$) in comparison to nicotine-withdrawal and vehicle only exposures. Daily sertraline treatment significantly diminished ($p < 0.001$) the nicotine-withdrawal triggered somatic signs in mice relative to mice that were exposed to nicotine-withdrawal and vehicle administration (Fig.

1). No significant change in somatic withdrawal signs was observed in mice that were injected lacidipine *per se* (3 mg/kg) and mice subjected to vehicle treatments.

Lacidipine decreased nicotine-withdrawal induced memory deficits in mice

In acquisition trials, no significant intergroup variation in day 13 TL of mice was noted in the EPM test [$F_{(7, 40)} = 2.061$, $p > 0.05$] (Fig. 2a). However, in retrieval trials of the EPM test, we observed that nicotine-withdrawal significantly ($p < 0.001$) increased the TL in rodents relative to vehicle-treated rodents [$F_{(7, 40)} = 18.18$, $p < 0.001$] (Fig. 2b). These results indicated that mice subjected to nicotine-withdrawal, after a sustained nicotine exposure for 7 days, displayed poor retention of previous days' learned task. Lacidipine (doses 1 and 3 mg/kg *b.w.*) treatment substantially decreased ($p < 0.05$; $p < 0.001$) nicotine-withdrawal triggered upsurge in TL in mice relative to mice that were exposed to nicotine-withdrawal and vehicles. Daily sertraline administration substantially ($p < 0.001$) reduced nicotine-withdrawal induced increase in TL in mice in relation to mice subjected to nicotine-withdrawal and vehicle treatments. Furthermore, Bay-K8644 (Ca^{2+} agonist) administration before trials negated ($p < 0.01$) the lacidipine (3 mg/kg) triggered diminution in TL in mice subjected to nicotine-withdrawal in comparison to mice that were exposed to nicotine-withdrawal and lacidipine (3 mg/kg) treatments. In acquisition or retrieval tests, no significant intergroup variation in TL of vehicle-treated or lacidipine *per se* (3 mg/kg) injected groups has been observed. However, a noteworthy decline ($p < 0.001$) in the 14^{th} day TL of vehicle treated group in comparison to 13^{th} day TL was noted in this study ($t = 10.13$, $df = 5$, $p < 0.0002$).

Lacidipine decreased nicotine-withdrawal induced anxiety-like symptoms in mice

In the mirror chamber test, a noteworthy ($p < 0.001$) increase in mirror-compartment entering latency [$F_{(7, 40)} = 24.56$, $p < 0.001$], and decline in number of entries [$F_{(7, 40)} = 17.19$, $p < 0.001$] and time expended in mirror-compartment [$F_{(7, 40)} = 95.06$, $p < 0.001$] was observed in mice that were subjected to nicotine-withdrawal in comparison to vehicle-treated control mice. A significant reduction in latency to enter mirror-compartment ($p < 0.05$; $p < 0.001$) (Fig. 3a), and an increase in number of entries ($p < 0.01$; $p < 0.001$) (Fig. 3b) and time expended in mirror-compartment ($p < 0.01$; $p < 0.001$) (Fig. 3c) was noted when lacidipine (1 and 3 mg/kg) was injected in animals subjected to nicotine-withdrawal with respect to animals that were provided nicotine-withdrawal and vehicle treatments only. Sertraline substantially ($p < 0.001$) lessened the nicotine-withdrawal triggered anxiety-like symptoms in mice in the mirror chamber test relative to mice subjected to nicotine-withdrawal and vehicle treatments. Administration of Bay-K8644 inhibited the anxiolytic activity of lacidipine (3 mg/kg) in rodents subjected to nicotine-withdrawal when juxtaposed to rodents that were exposed to nicotine-withdrawal and lacidipine (3 mg/kg) treatments. Bay-K8644 significantly lessened the lacidipine (3 mg/kg) induced reduction in mirror-compartment entering latency ($p < 0.05$) and upsurge in the entry numbers ($p < 0.001$) and time expended in the mirror-compartment ($p < 0.001$) in mice subjected to nicotine-withdrawal. No statistically significant difference in parameters quantified in the mirror chamber test was detected between the vehicle control and lacidipine (3) groups.

Lacidipine decreased nicotine-withdrawal induced upsurge in the immobility duration in mice

Mice subjected to nicotine-withdrawal, after continued exposure to nicotine for 7 days, manifested a significant ($p < 0.001$) rise in the immobility period (s) in TST relative to vehicle-treated mice. Administration of lacidipine (doses 1 and 3 mg/kg) for 14 days substantially decreased ($p < 0.05$; $p < 0.01$) the nicotine-withdrawal triggered escalation in the duration of immobility in mice when juxtaposed to mice that were exposed to nicotine-withdrawal and drug-vehicles only [$F_{(7, 40)} = 16.32$, $p < 0.001$]. Sertraline markedly ($p < 0.001$) decreased the immobility period in mice that were subjected to nicotine-withdrawal relative to mice that were given nicotine-withdrawal and vehicle treatments. Bay-K8644 (Ca^{2+} agonist) substantially ($p < 0.05$) diminished the lacidipine (3 mg/kg) induced decrease in immobility period in rodents that were subjected to nicotine-withdrawal relative to rodents that were given nicotine-withdrawal and lacidipine (3 mg/kg) treatments. There was no significant change in the immobility duration of mice treated with lacidipine *per se* (3 mg/kg) relative to mice that were injected drug-vehicle only (Fig. 4).

Lacidipine decreased nicotine-withdrawal induced lipid peroxidation in the brain

Results showed that nicotine-withdrawal triggered a noteworthy ($p < 0.001$) increase in the brain TBARS levels when juxtaposed to vehicle only treatment. Treatment with lacidipine (1 and 3 mg/kg) daily for 14 days significantly reduced ($p < 0.05$; $p < 0.001$) the nicotine-withdrawal induced upsurge in the brain TBARS content in comparison nicotine-withdrawal and vehicle exposures [$F_{(7, 40)} = 54.68$, $p < 0.001$]. Administration of sertraline significantly declined ($p < 0.001$) the TBARS levels relative to vehicle treatment in separate groups of animals subjected to nicotine-withdrawal. Bay-K8644 markedly attenuated ($p < 0.001$) the lacidipine (3 mg/kg) triggered decrease in lipid peroxidation in the brain of animals that were subjected to nicotine-withdrawal relative to animals that were exposed to nicotine-withdrawal and lacidipine (3 mg/kg). Comparing the vehicle control group and the lacidipine (LCD 3) group disclosed statistical insignificant disparity of brain TBARS (mean) levels (Fig. 5a).

Lacidipine enhanced the brain GSH level in mice subjected to nicotine-withdrawal

A substantial ($p < 0.001$) decrease in the brain reduced GSH levels was observed in mice subjected to nicotine-withdrawal when juxtaposed to mice that were provided drug-vehicle only. However, a significant elevation ($p < 0.01$; $p < 0.001$) in the brain GSH levels was noted when lacidipine (doses 1 and 3 mg/kg *b.w.*) was administered for 14 days daily in comparison to exposure to vehicle for same duration in separate groups of mice subjected to nicotine-withdrawal [$F_{(7, 40)} = 117.2$, $p < 0.001$]. Sertraline treatment significantly ($p < 0.001$) attenuated nicotine-withdrawal-induced diminution in the brain GSH concentrations in mice relative to mice that were given nicotine-withdrawal and vehicle treatments. Bay-K8644 prohibited ($p < 0.001$) the lacidipine (3 mg/kg) triggered intensification of brain GSH concentrations in mice that were subjected to nicotine-withdrawal relative to mice that received nicotine-withdrawal and lacidipine (3 mg/kg) treatments (Fig. 5b). There was no significant change in brain GSH levels of lacidipine *per se* and vehicle-treated mice.

Lacidipine inhibited nicotine-withdrawal induced decline in the brain SOD activity

Animals subjected to sudden nicotine-withdrawal, after incessant exposure to nicotine for 7 days, manifested a significant ($p < 0.001$) decrease in the brain SOD activity when juxtaposed to vehicle-treated animals. Daily systemic administration of lacidipine (doses 1 and 3 mg/kg *b.w.*) for 14 days substantially attenuated ($p < 0.05$; $p < 0.001$) the nicotine-withdrawal triggered decrease in the brain SOD activity in mice when juxtaposed to mice that were subjected to nicotine-withdrawal and vehicle treatments alone [$F_{(7, 40)} = 88.62$, $p < 0.001$]. Sertraline noticeably ($p < 0.001$) enhanced the activity of brain SOD in mice that were subjected to nicotine-withdrawal relative to mice that were given nicotine-withdrawal and vehicle treatments. Bay-K8644 (Ca^{2+} agonist) substantially ($p < 0.001$) diminished the lacidipine (3 mg/kg) triggered escalation in activity of brain SOD in mice that were subjected to nicotine-withdrawal *vis-a-vis* to mice that were put through nicotine-withdrawal and lacidipine (3 mg/kg) treatments. Comparing the vehicle control group and the lacidipine (3) group disclosed statistical insignificant disparity of brain SOD activity (Fig. 5c).

Lacidipine reduced nicotine-withdrawal triggered waning of the brain catalase activity

Nicotine-withdrawal triggered a noteworthy ($p < 0.001$) decrease in the enzymatic activity of CAT when compared with drug-vehicle treatment. Treatment with lacidipine (doses 1 and 3 mg/kg *b.w.*) for 14 days substantially attenuated ($p < 0.05$; $p < 0.001$) the nicotine-withdrawal triggered decrease in the brain CAT enzymatic activity in comparison to nicotine-withdrawal and vehicle treatments only in mice [$F_{(7, 40)} = 149.4$, $p < 0.001$]. Administration of sertraline significantly enhanced ($p < 0.001$) the CAT activity in relation to drug-vehicle treatments in the brain of separate groups of mice that were given nicotine-withdrawal exposure. Bay-K8644 (Ca^{2+} agonist) substantially ($p < 0.001$) lessened the lacidipine (3 mg/kg) induced upsurge in the brain CAT activity in mice that were subjected to nicotine-withdrawal relative to mice that were provided nicotine-withdrawal and lacidipine (3 mg/kg) treatments. Comparing the vehicle control group and the lacidipine (3) group disclosed statistical insignificant disparity of brain CAT activity (Fig. 5d).

Lacidipine decreased the brain nitrite content in mice subjected to nicotine-withdrawal

A substantial ($p < 0.001$) upsurge in the nitrite levels was observed in mice that were subjected to nicotine-withdrawal when juxtaposed to mice that were systemically with drug-vehicles. However, a significant decline ($p < 0.01$; $p < 0.001$) in the brain nitrite levels was noted when lacidipine (1 and 3 mg/kg) was administered for 14 days daily in mice subjected to nicotine-withdrawal in comparison to mice given nicotine-withdrawal and vehicle treatments only [$F_{(7, 40)} = 215.5$, $p < 0.001$]. Administration of sertraline significantly decreased ($p < 0.001$) the nitrite levels in comparison to vehicle treatments in the brain of separate groups of mice that were exposed to nicotine-withdrawal. Bay-K8644 (Ca^{2+} agonist) reduced ($p < 0.001$) the lacidipine (3 mg/kg) induced decrease in brain nitrite level in mice that were subjected to nicotine-withdrawal relative to mice that were given nicotine-withdrawal and lacidipine (3

mg/kg) treatments. Comparing the vehicle control group and the lacidipine (3) group disclosed statistical insignificant disparity of brain nitrite contents (Fig. 5e).

Discussion

In the present study, nicotine administration was ceased after a continuous exposure to nicotine for 7 days to elicit withdrawal behavior in mice. Nicotine-withdrawal instigated somatic withdrawal signs and affective symptoms including aberrations in memory functions in mice. Alongside administration of nicotine for 7 days, lacidipine (0.3, 1, and 3 mg/kg) was given from 1st day to 14th day daily in mice to determine the repercussions of LCD on nicotine-withdrawal triggered behavioral aberrations in mice. In previous studies, the development, expression, and progress of nicotine-withdrawal signs have been associated with different subtypes of LTCCs (Katsura et al. 2002; Liu et al. 2017; Bernardi et al. 2014) that prompted to use of LCD treatment protocol along with the nicotine-withdrawal paradigm in the current study. Suppression of initial involvement of Ca(v)1.3 subtype of LTCCs by Ca^{2+} channel blockers during the nicotine exposure might attenuate progressive participation of other LTCC subtypes such as Ca(v)1.2 channels in the expression of withdrawal symptoms after nicotine-withdrawal (Bernardi et al. 2014). We observed that nicotine-withdrawal triggered a range of brain abnormalities *e.g.*, somatic withdrawal symptoms, loss of memory, and symptoms of anxiety and depression in mice. Lacidipine (dose 1 and 3 mg/kg, *i.p.*) treatment reduced the nicotine-withdrawal associated behavioral symptoms (both somatic and affective) and also decreased the oxidative and nitrite burden in the brain of rodents used. These effects of LCD (L-type Ca^{2+} antagonist) were significantly suppressed by treatment of mice with Bay-K8644 (L-type Ca^{2+} agonist) prior to behavioral experiments. These findings indicated that LCD mitigated nicotine-withdrawal-associated brain dysfunctions by restoration of cellular Ca^{2+} equilibrium. Furthermore, in the present study, mice of both sexes were used. However, statistical analysis of the data showed that sex of mice as a variable has no influence on the outcomes of different behavioral and biochemical parameters studied in the current research (refer to supplementary data file).

Somatic nicotine-withdrawal signs were determined 48 h after abrupt suspension of nicotine treatment in mice that received LCD or vehicle. Mice subjected to nicotine-withdrawal showed a significant increase in somatic symptoms such as grooming, genital licking, rearing, jumping, leg licking, and head shaking. Lacidipine (dose 1 and 3 mg/kg) or sertraline (standard drug) treatments (*i.p.*) abolished the appearance of somatic withdrawal symptoms in mice that had been subjected to nicotine-withdrawal after sustained exposure to nicotine for 7 days. Decrease in somatic symptoms of nicotine-withdrawal by lacidipine (3 mg/kg) was akin to sertraline that confirmed the efficacy of LCD (3 mg/kg) against nicotine-withdrawal induced symptoms.

All the groups were subjected to behavioral tests of memory impairment, anxiety, and depression (affective signs). In the EPM test, retention of memory 24 h after acquisition or training trials was tested by measuring the transfer latency (TL) (Bansal and Parle 2011). Mice that were subjected to nicotine-withdrawal showed an increase in time taken to enter the closed arm (TL) in retrieval trials. The findings showed loss of memory in mice subjected to nicotine-withdrawal. Comparison of day 13 and 14 TL of

vehicle control groups showed a substantial decrease in the day 14 TL of vehicle control group relative to day 13 TL that validated the EPM memory test. Symptoms of anxiety in mice were assessed using the mirror chamber test. Mice subjected to nicotine-withdrawal, after continuous exposure to nicotine for 7 days, showed aversion to the mirror chamber. These results indicated that nicotine-withdrawal triggered symptoms of anxiety in mice. Rodents have an inherent penchant for closed spaces and they habitually avoid large open spaces that form the basis of the EPM memory test (Itoh et al. 1990). Although, nicotine-withdrawal induced rise in anxiety-like symptoms might intensify the urge in mice to locate the closed space, however, in the EPM test mice failed to enter the closed arm at the earliest in response to nicotine-withdrawal, which was evident by an increase in TL. Hence, the results of the EPM test also substantiated findings of the mirror chamber test in which mice subjected to nicotine-withdrawal avoided the mirror chamber and depicted severe anxiety-levels in this study. It can be inferred that nicotine-withdrawal triggered loss of memory and anxiety-like symptoms in mice. Furthermore, an upsurge in the immobility period in TST indicated signs of depression in mice that were put through nicotine-withdrawal. Daily treatment with lacidipine (1 and 3 mg/kg) caused a substantial drop in anxiety- and depression-like symptoms, and an increase in spatial memory of mice that were subjected to nicotine-withdrawal. Lacidipine (1 and 3 mg/kg) diminished the nicotine-withdrawal triggered reluctance of mice towards the mirror chamber. A significant diminution of mean TL value in EPM paradigm and immobility period (s) in TST was deciphered in mice subjected to nicotine-withdrawal and lacidipine (1 and 3 mg/kg) treatments. In previous studies, depreciation of physical (somatic signs) and affective nicotine-withdrawal signs (anxiety, depression, and memory deficits) by calcium channel blockers emphasize the contribution of LTCCs in the progress of physical dependence on nicotine and manifestation of withdrawal signs after inhibition of further exposure to nicotine (Jackson and Damaj 2009; Biala et al. 2014). Sertraline (SSRI), employed as a standard drug in the existing study, attenuated the expression of somatic and affective withdrawal signs of nicotine in mice. We observed that improvement in affective signs of nicotine-withdrawal by lacidipine (dose 3 mg/kg) was comparable to sertraline. The existing literature substantiates considerable improvement in nicotine withdrawal triggered anxiety, depression, and mental disorders in addicted humans by SSRIs through a rise in serotonin and dopamine levels in the brain (Harrison et al. 2001). Furthermore, the findings showed that a decrease in behavioral abnormalities or affective nicotine-withdrawal signs (*e.g.*, symptoms of anxiety, depression, and memory loss) by lacidipine (3 mg/kg) in mice subjected to nicotine-withdrawal was significantly decreased by Ca^{2+} agonist, Bay-K8644.

Existing evidence indicate that LTCCs facilitate early activities of nicotine and also depict a vital function in long-term molecular and behavioral changes after termination of nicotine exposure (Biała et al. 2014; Katsura et al. 2002). Furthermore, nicotine-triggered increase in Ca^{2+} influx through nAChRs (Changeux 2011) can augment several Ca^{2+} dependent cytotoxic mechanisms (Dong et al. 2009). Ca^{2+} induced ROS spontaneously react with lipids and proteins that perpetuate the production of neurotoxic aldehydes (*e.g.*, acrolein, 4-hydroxy 2-nonenal, isoprostanes), alcohols, and carbonyls. Malondialdehyde (MDA) is a lipid peroxidation neurotoxicant determined by measuring TBARS levels. MDA forms an adduct with biomolecules, enhances the stability of neurotoxic (*e.g.*, advanced glycation end-products) aggregates,

and accentuates a higher immune response by stimulation of glia and astrocytes. In the present study, an abrupt nicotine-withdrawal, after sustained exposure to nicotine for 7 days, resulted in a significant increase in lipid peroxidation (TBARS), total nitrite levels, and decrease in the endogenous antioxidants (GSH, SOD, and catalase) in the brain of mice. Pathogenic increase in nitric oxide by Ca^{2+} ions hasten the peroxynitrite radical formation, nitrosylation of proteins, and activation of the neuro-inflammatory cascade (Picon-Pages et al. 2019). Previous studies also support that prolonged nicotine exposure and a sudden withdrawal culminate oxidative stress accompanied by brain atrophy (Newman et al. 2002; Hritcu et al. 2009). Several factors such as higher polyunsaturated fatty acids, oxygen consumption, transition metals, and relatively lower antioxidants predispose the brain to oxidative neurodegeneration (Halliwell and Guttridge 1988). However, the present findings showed that treatment with lacidipine (1 and 3 mg/kg) substantially depressed lipid peroxidation in the brain of mice that were subjected to nicotine-withdrawal.

Glutathione (GSH) is a tripeptide required as a cofactor for many enzymes (*e.g.*, glutathione peroxidase, glutathione reductase, glutathione *S*-transferase), which are involved in the detoxification of free radicals and lipid peroxidation products. Catalase and superoxide dismutase (SOD) are antioxidants, which directly scavenge free radicals such as hydroxyl radicals, hydrogen peroxide, and superoxide anions. A significant decrease in these endogenous antioxidants in the brain of mice subjected to continued nicotine exposure and nicotine-withdrawal has been observed in earlier studies (Hritcu et al. 2009). In this study also, we noticed that nicotine-withdrawal, after nicotine exposure for 7 days, manifested a significant reduction in the antioxidants (GSH, SOD, and CAT) in the brain of mice. The antioxidants such as GSH level, SOD, and catalase activities were substantially improved by lacidipine (1 and 3 mg/kg for 14 days) in mice that were subjected to nicotine-withdrawal. Findings from previous studies also support the potent antioxidant effects of LCD in the brain (Cominacini et al. 2003; Khurana and Bansal 2019a). Sertraline was used for standard antidepressant treatment in this protocol. Sertraline is a selective serotonin reuptake inhibitor (SSRI) clinically used to treat depression, panic, anxiety, or obsessive-compulsive symptoms (Shoaib and Buhidma 2018). Administration of sertraline in mice subjected to nicotine-withdrawal, after 7 days exposure to nicotine, significantly alleviated the rise in brain oxido-nitrosative stress, and also improved the endogenous antioxidant activities. Earlier findings also suggest antioxidative activities of sertraline in the brain and plasma (Michalakeas et al. 2011; Abdel Salam et al. 2013). The current findings corroborated that the anti-oxidative effects of lacidipine (3 mg/kg) were comparable to sertraline against nicotine-withdrawal. To explicate the part of Ca^{2+} channels, Bay-K8644 (L-type Ca^{2+} agonist) was injected in lacidipine (3 mg/kg) treated mice that were subjected to nicotine-withdrawal after exposure to nicotine for 7 days. Administration of Bay-K8644 before behavioral studies significantly negated the antioxidant activities of lacidipine (3 mg/kg) in the brain of mice against nicotine-withdrawal. These outcomes aptly suggested that suppression of L-type Ca^{2+} activity by LCD is attributed to the observed lowering of oxido-nitrosative stress and fortification of endogenous antioxidants in mice subjected to nicotine exposure and withdrawal (Fig. 6). Furthermore, the chemical structure of LCD reveals the presence of at least 4 hydrogen (–H) acceptor sites and one hydrogen donor site. According to the classical structure-activity relationship (SAR) theory, these –H acceptor and donor

groups impart free radical scavenging property to LCD and helps in terminating the self-propagating chain reaction of free radicals.

Conclusion

It can be inferred that nicotine-withdrawal symptoms were decreased by lacidipine owing to the inhibition of Ca^{2+} influx and antioxidant activities in the brain. Lacidipine alone or in combination with other prescription medications (*e.g.*, bupropion or varenicline) can mitigate the biochemical changes that occur during initial nicotine exposure and subsequently suppress the unwanted nicotine-withdrawal symptoms.

Abbreviations

ANOVA: Analysis of variance

b.w.: Body weight

CAT: Catalase

DA: Dopamine

EPM: Elevated plus maze

GABA: Gamma amino butyric acid

GSH: Glutathione

i.p.: Intraperitoneal

LCD: Lacidipine

LTCCs: L-type voltage-dependent Ca^{2+} channels

MDA: Malondialdehyde

NAc: Nucleus accumbens

nAChRs: Nicotinic cholinergic receptors

Nic: Nicotine

O.D.: Optical density

ROS: Reactive oxygen species

s.c.: Subcutaneous

SOD: Superoxide dismutase

t.i.d.: Ter in die (3 times a day)

TBARS: Thiobarbituric acid reactive substances

TL: Transfer latency

TST: Tail suspension test

VTA: Ventral tegmentum

λ_{max}: Maximum wavelength

Declarations

Acknowledgements

The authors are thankful to I. K. Gujral Punjab Technical University, Kapurthala (Punjab) and the management of A.S.B.A.S.J.S.M. College of Pharmacy, Bela (Ropar) for providing necessary research facilities.

Funding

The present research received no specific funding.

Competing interests

The authors declare no competing interest.

Availability of data and materials

The data are available.

Authors' contribution

NB conceived and designed this study. KK performed the research. MK analyzed the data, drafted the original and revised manuscript. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Compliance with ethical standards

This study was approved by the "Institutional Animal Ethics Committee (IAEC)" of the A.S.B.A.S.J.S.M. College of Pharmacy, Bela (Ropar) vide protocol no. ASCB/IAEC/08/15/108 (dated: 28-11-2015). The guidelines of "The Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA)," Ministry of Forests & Environment, and Government of India (New Delhi) were followed for all animal experiments and care of animals.

Consent to participate

Not applicable.

Consent to publish

All authors have agreed to publish this study.

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Figures

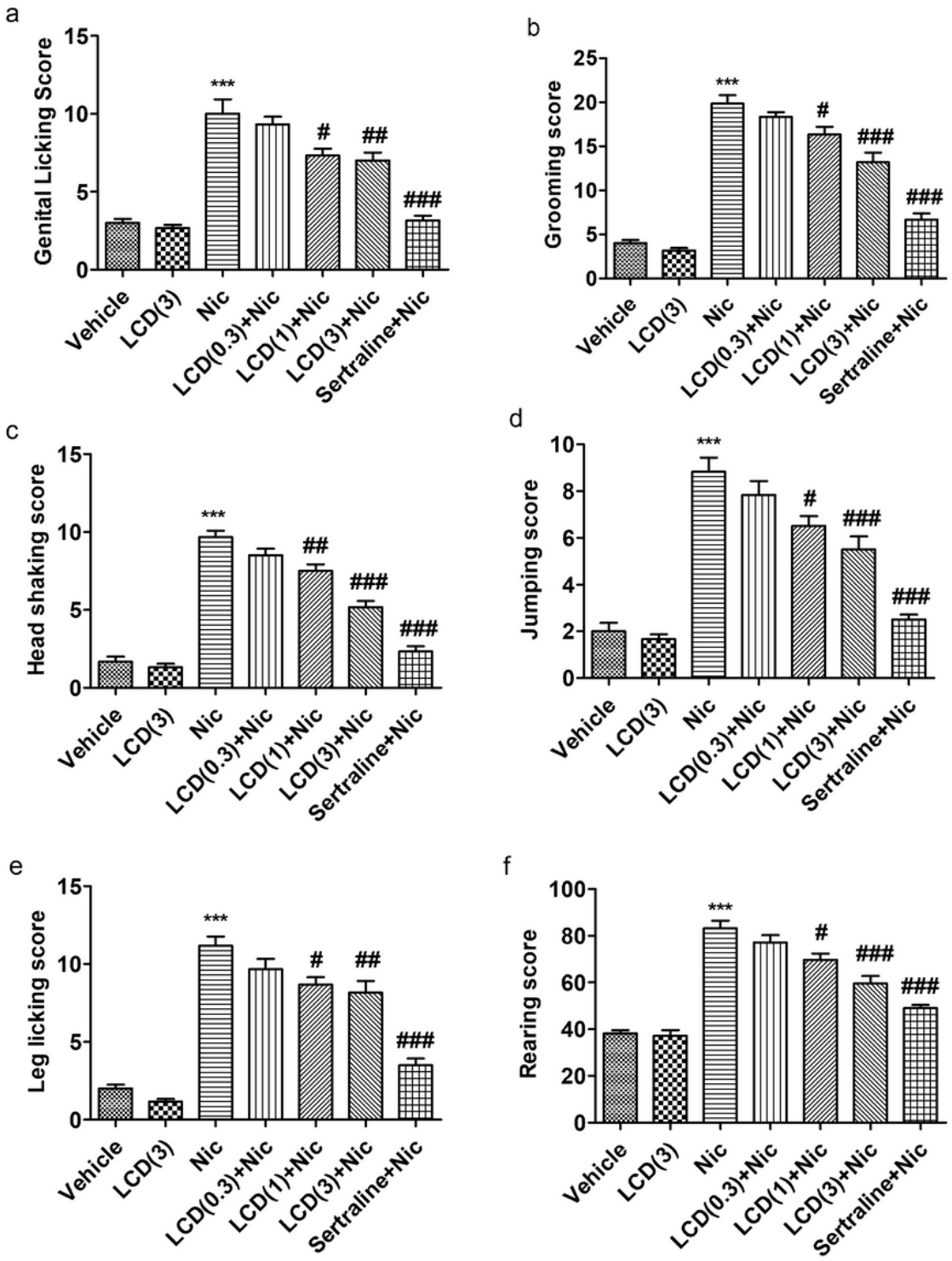

Figure 1

Lacidipine (LCD; dose 0.3, 1, and 3 mg/kg) treatment mitigated somatic withdrawal signs in mice after sudden termination of exposure to (−)-nicotine hydrogen tartrate (Nic). Nic (dose 3.35 mg/kg, t.i.d.) was administered from day 1 to 7 and thereafter, Nic administration was discontinued to trigger somatic withdrawal symptoms that were measured on day 9. Statistical analysis of somatic withdrawal signs a Genital licking score, b Grooming score, c Head shaking score, d Jumping score, e Leg licking score, and f

Rearing score using one-way ANOVA followed by Tukey's HSD post-hoc test. All the results are expressed as mean ± S.E.M. (n = 6). Significance at *** p < 0.001 vs. vehicle control group, # p < 0.05, ## p < 0.01, ### p < 0.001 vs. Nic group.

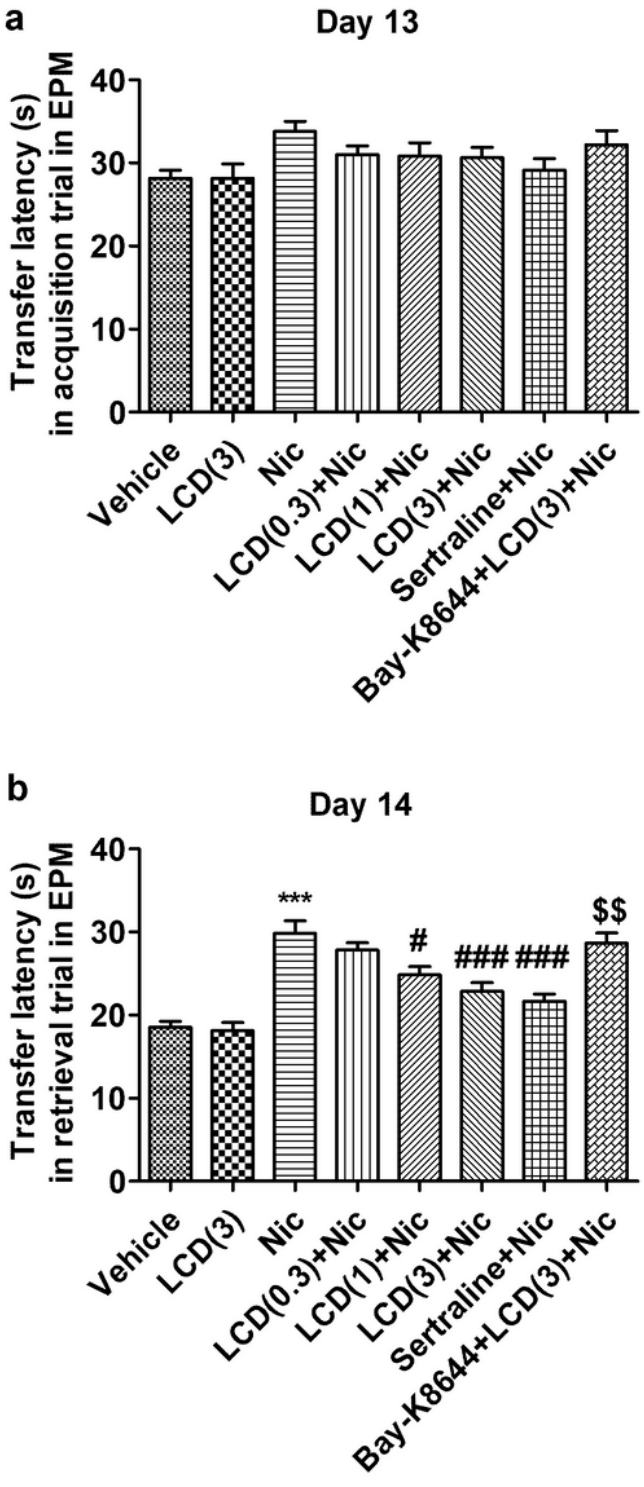

Figure 2

Treatment with lacidipine (LCD; dose 0.3, 1, and 3 mg/kg) alleviated memory deficits in mice that were exposed to (−)-nicotine hydrogen tartrate (Nic, dose 3.35 mg/kg, t.i.d.) from day 1 to 7 and thereafter, Nic

administration was discontinued. Bay-K8644 decreased memory enhancing effects of LCD in mice subjected to Nic withdrawal. Statistical analysis of a mean transfer latency (s) during acquisition trial on day 13, and b mean transfer latency (s) during retrieval trial on day 14 in elevated plus maze (EPM) test using one-way ANOVA followed by Tukey's HSD post-hoc test. All the results are expressed as mean ± S.E.M. (n = 6). Significance at *** p < 0.001 vs. vehicle control group, # p < 0.05, ### p < 0.001 vs. Nic group, $$ p < 0.01 vs. LCD(3)+Nic group.

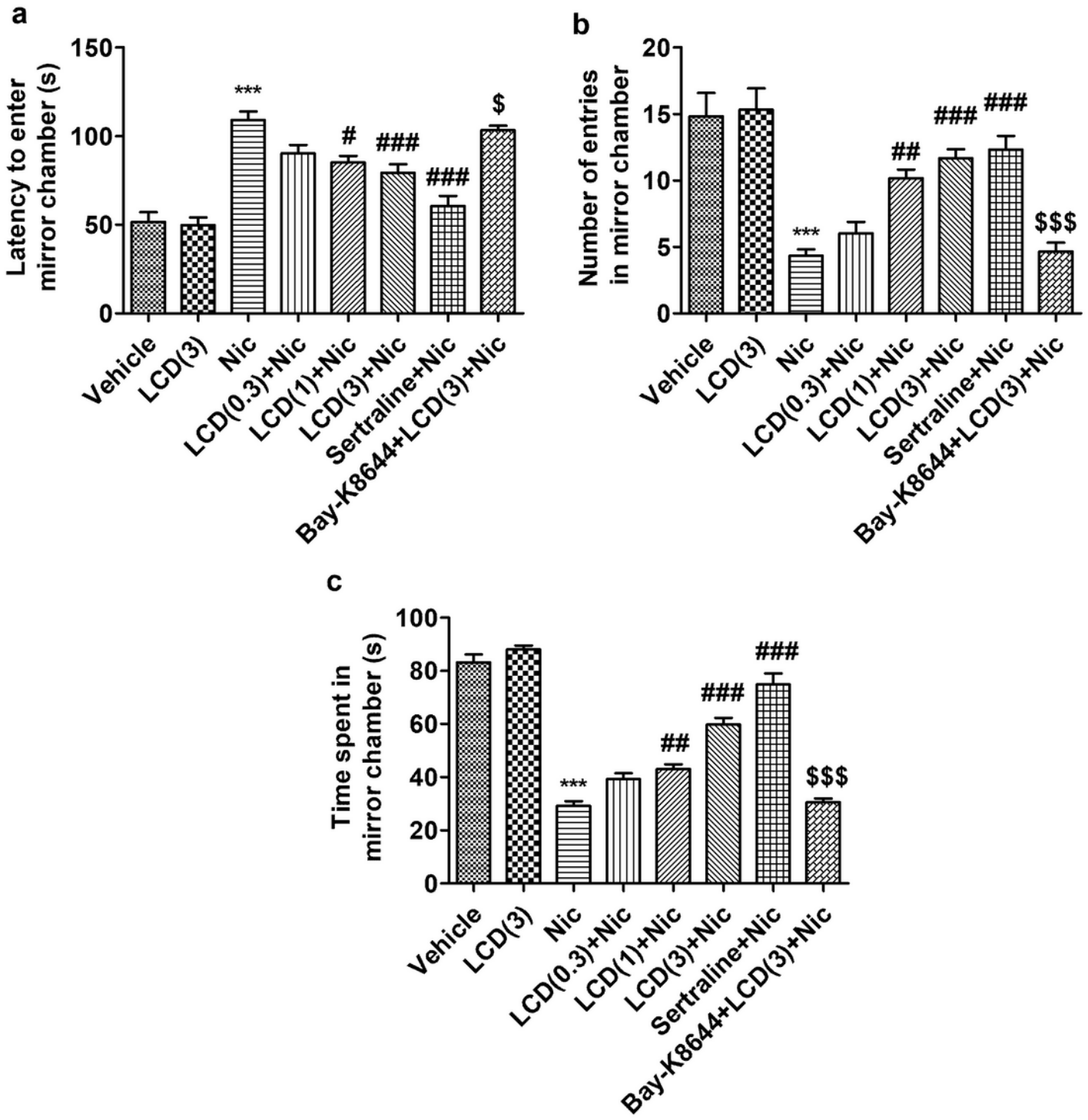

Figure 3

Lacidipine (LCD; 0.3, 1 and 3 mg/kg) treatment decreased nicotine-withdrawal induced anxiety-like symptoms in mirror chamber apparatus test in mice that were exposed to (−)-nicotine hydrogen tartrate (Nic, dose 3.35 mg/kg, t.i.d.) from day 1 to 7 and thereafter, Nic administration was discontinued. Bay-K8644 decreased anti-anxiety like effects of LCD in mice subjected to Nic withdrawal. Statistical analysis of a Latency to enter mirror chamber (s), b Number of entries in mirror chamber, and c Time spent in mirror chamber using one-way ANOVA followed by Tukey's HSD post-hoc test. All the results are expressed as mean ± S.E.M. (n = 6). Significance at *** $p < 0.001$ vs. vehicle control group, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. Nic group, $ $p < 0.05$, $$$ $p < 0.001$ vs. LCD(3)+Nic group.

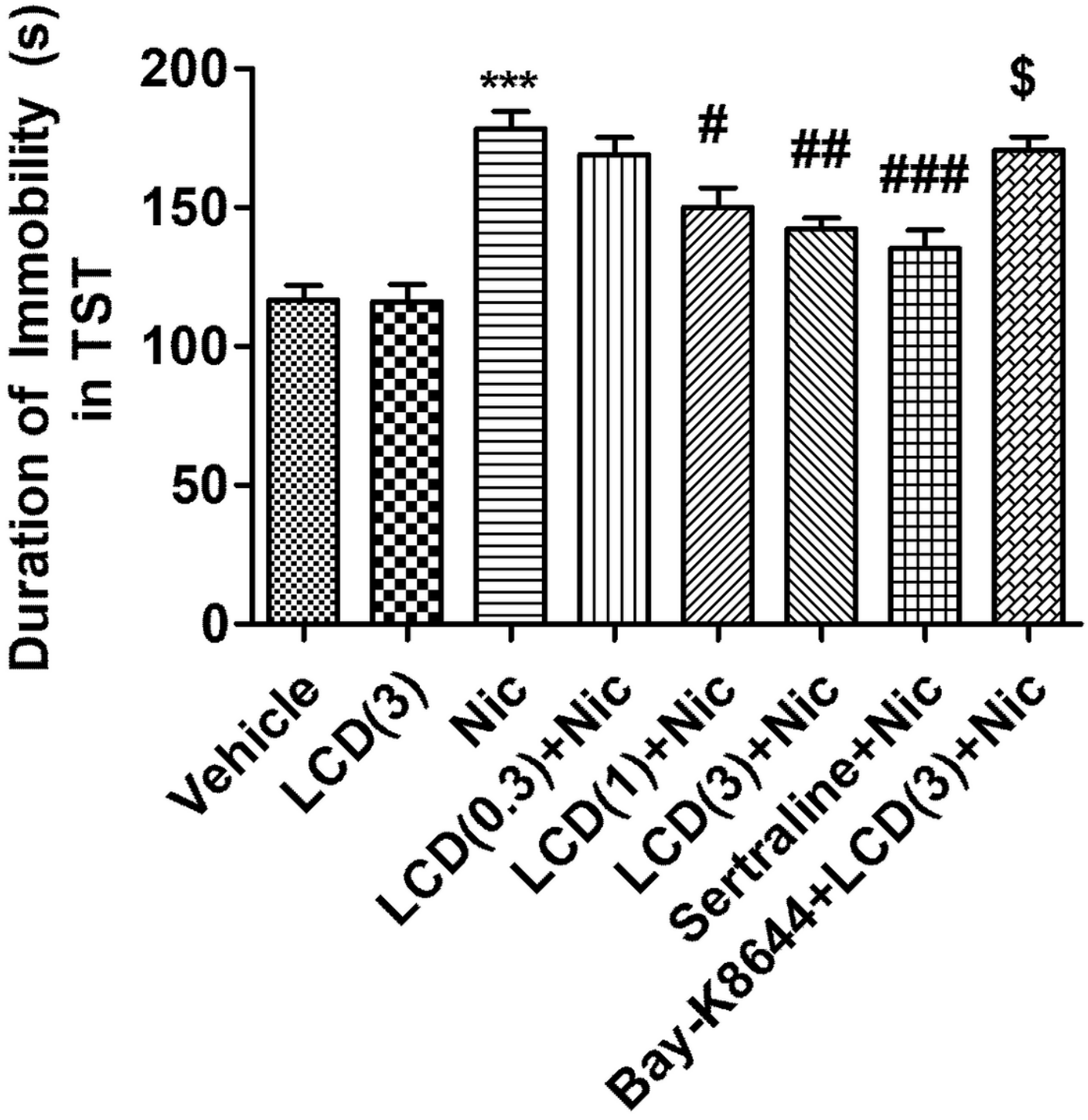

Figure 4

Lacidipine (LCD; 0.3, 1 and 3 mg/kg) treatment for 14 days reduced nicotine withdrawal induced symptoms of depression in mice that were exposed to (−)-nicotine hydrogen tartrate (Nic, dose 3.35 mg/kg, t.i.d.) from day 1 to 7 and thereafter, Nic administration was discontinued. Bay-K8644 decreased anti-depressive like effects of LCD in mice subjected to Nic withdrawal. Statistical analysis of immobility period (s) in tail suspension test (TST) was accomplished using one-way ANOVA followed by Tukey's HSD post-hoc test. All the results are expressed as mean ± S.E.M. (n = 6). Significance at *** $p < 0.001$ vs. vehicle control group, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. Nic group, $ $p < 0.05$ vs. LCD(3)+Nic group.

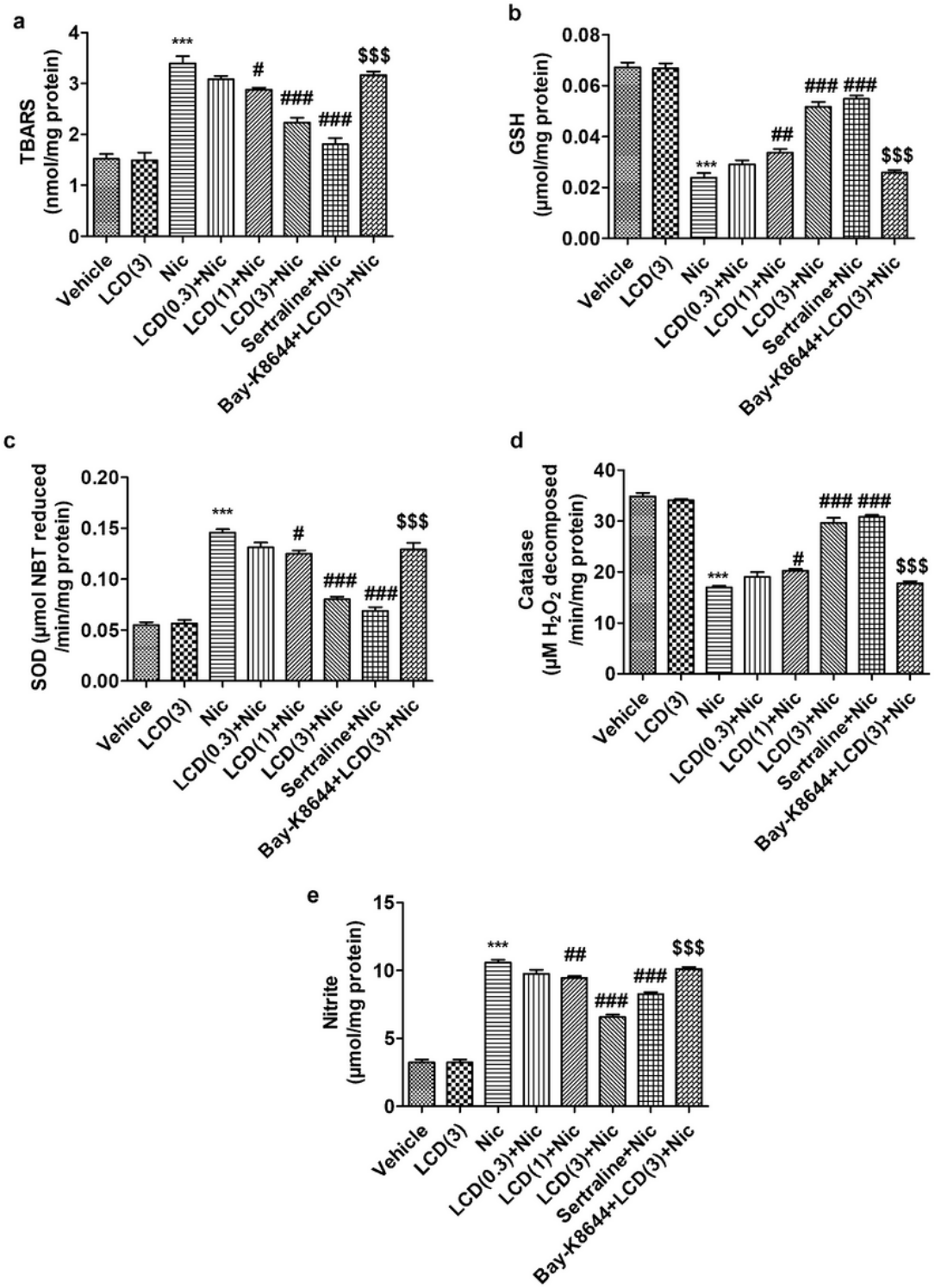

Figure 5

Treatment with lacidipine (LCD; 0.3, 1 and 3 mg/kg) for 14 days daily decreased the nicotine withdrawal induced oxido-nitrosative stress in the brain of mice that were exposed to (−)-nicotine hydrogen tartrate (Nic, dose 3.35 mg/kg, t.i.d.) from day 1 to 7 and thereafter, Nic administration was discontinued. Bay-K8644 decreased anti-oxidant and anti-nitrative effects of LCD in mice subjected to Nic withdrawal. Statistical analysis of brain a Thiobarbituric acid reactive substances (TBARS), b Reduced glutathione

(GSH), c Superoxide dismutase (SOD), d Catalase, and e Nitrite content was accomplished using one-way ANOVA followed by Tukey's HSD post-hoc test. All the results are expressed as mean ± S.E.M. (n = 6). Significance at *** $p < 0.001$ vs. vehicle control group, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. Nic group, $$$ $p < 0.001$ vs. LCD(3)+Nic group.

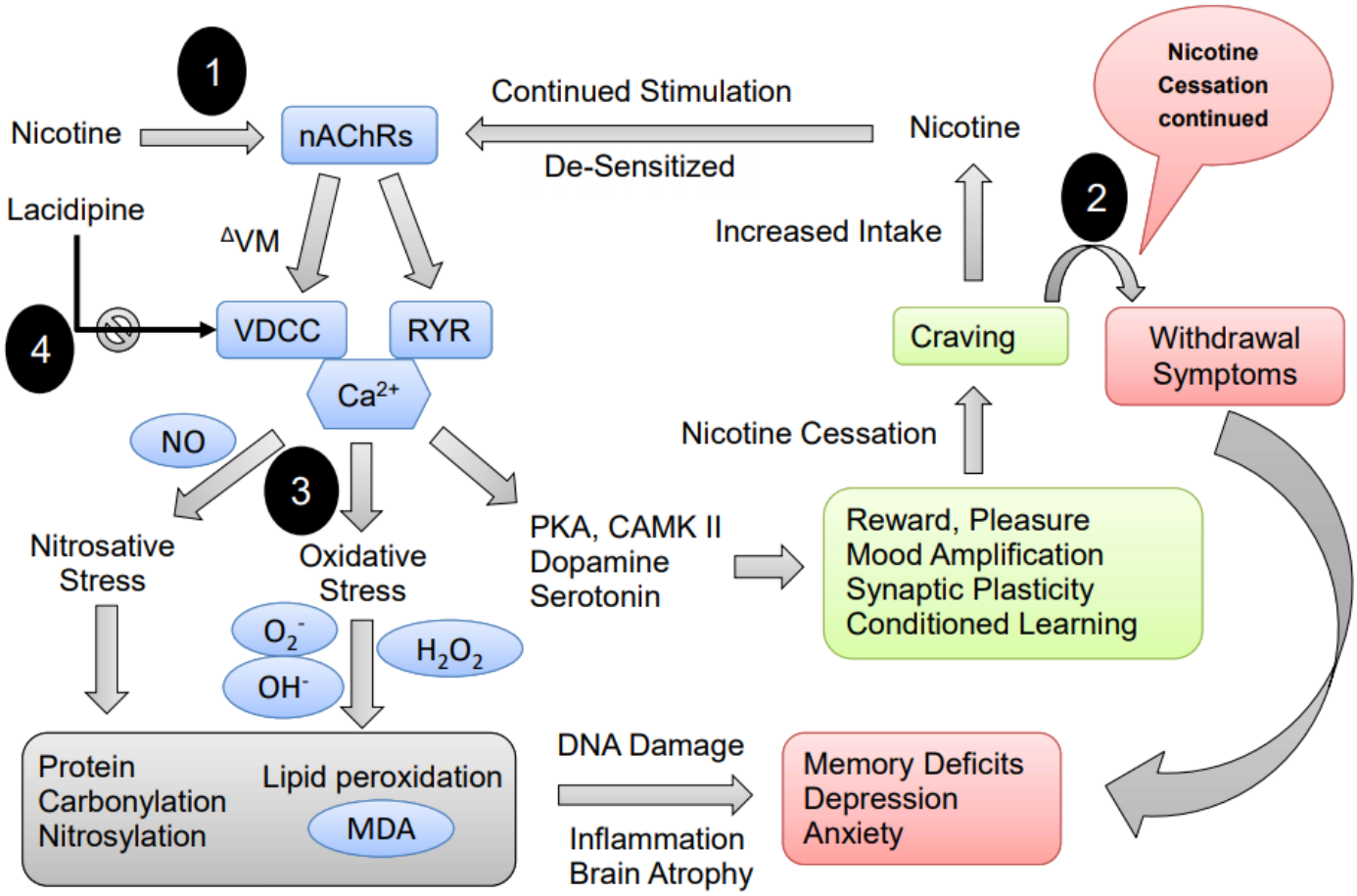

Figure 6

Possible mechanism of lacidipine induced mitigation of nicotine-withdrawal triggered behavioral abnormalities in mice. 1) Exposure to nicotine activates nicotinic acetylcholine receptors (nAChRs) that leads to rise in intracellular calcium (Ca2+) levels through L-type voltage-dependent Ca2+ channels (VDCCs) and ryanodine receptors (RYR). Cytoplasmic increase in Ca2+ activates several second messengers such as protein kinase A and C (PKA, PKC), and Ca2+/calmodulin-dependent protein kinase II (CAMK II), and release neurotransmitters (e.g., dopamine, serotonin) in the brain. These Ca2+ dependent pathways are believed to play important roles in cognition, behavior, and other brain functions. An increase in dopamine at nucleus accumbens (NAc) imparts reward and pleasurable effects. Cessation of nicotine exposure precipitates craving behavior in rodents. Re-exposure to nicotine satisfies the nicotine-craving, however, the nicotine consumption is increased substantially over long-durations due to

desensitization of nAChRs. 2) Nicotine withdrawal after continuous exposure to nicotine precipitates behavioral abnormalities such as abstinence signs (grooming, genital licking, rearing, jumping, leg licking and head shaking), loss of cognition, depressive and anxiety-like behavior. 3) Chronic exposure to nicotine exacerbates oxidative stress through Ca2+ dependent pathways. High Ca2+ activity causes mitochondrial dysfunction, hastens ROS and peroxynitrite output via Ca2+ mediated inducible nitric oxide synthase (iNOS) activation, and lipid peroxidation. Ca2+ hyper stimulates brain immune cells (e.g., microglia, astrocytes), enhances expression of pro-inflammatory cytokines (e.g., TNF-α, interleukins), and activates apoptotic and necrotic cell death pathways. Ca2+ mediated neurodegeneration underlies many brain dysfunctions (e.g., cognitive decline, symptoms of depression and anxiety) and brain atrophy. 4) Lacidipine (L-type Ca2+ channel antagonist) abolishes the oxidative stress, inflammation, and associated brain abnormalities by inhibiting the pathogenic increase in cytoplasmic Ca2+ levels and free radical scavenging activity owing to presence of hydrogen acceptor and donor groups in its molecular structure. Inhibition of chronic increase in Ca2+ levels by exposure to nicotine by lacidipine alleviates the nicotine withdrawal symptoms.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryData.xlsx