INTRODUCTION

Morphine, an opioid analgesic, has been used widely for relieving moderate to severe pain.[1] However, repeated administration results in development of tolerance and dependence.[2] This dependence may be precipitated as a consequence of unpleasant withdrawal reactions characterized by anxiety, agitation, insomnia, and diarrhea, on sudden stoppage of the drug or when morphine antagonist such as naloxone is administered. To avoid these adverse withdrawal reactions, there is a compulsion to continue the intake of the drug which may further reinforce addictive behavior.[3] Numerous reports have mentioned the role of calcium ions in morphine dependence as opioid receptors are functionally coupled to voltage-sensitive calcium channel (VS SCC). Acute administration of morphine decreases the intracellular calcium concentration by closing the VS SCC.[4] However, chronic usage induces an adaptive response resulting in an increase in basal-free calcium concentration in the neurons of central nervous system.[5,6] Administration of naloxone, a morphine antagonist, can cause a sharp increase in synaptosomal calcium concentration that may be responsible for sudden release of large amount

Objective: To observe the effect of L-type calcium channel blocker like nimodipine on morphine’s withdrawal when it was administered continuously along with morphine versus a single bolus dose of nimodipine, which was administered at the end of the experiment before the precipitation of withdrawal reaction in morphine-dependent rats. Materials and Methods: Four groups of adult male Wistar rats were rendered morphine dependent by subcutaneous injections of morphine at a dose of 10 mg/kg for 10 days. Nimodipine 10 mg/kg intraperitoneally (ip) administered to one group once daily before morphine administration in the entire experimental period, and another group received nimodipine only once at the end of the experiment as a single bolus dose 2 mg/kg before the administration of naloxone. Naloxone 3 mg/kg was administered ip to all the groups to precipitate withdrawal reactions. The withdrawal reactions were evaluated and scored as per the Gellert and Holtzman global withdrawal rating scale. Results: Nimodipine when administered as a single bolus dose before naloxone administration in morphine-dependent rats reduced the features of withdrawal reactions more effectively than continuous administration of nimodipine along with morphine throughout the experimental period. Conclusion: We discovered that nimodipine helps in attenuating the severity of morphine withdrawal having potential role encountered during pharmacotherapy with morphine management of opioid dependence, well memory, impairment, cell signaling and phosphorylation of neuron.

Keywords: Dependence and withdrawal reactions, morphine, naloxone, nimodipine
of neurotransmitters mediating the features of withdrawal reactions.[9] In view of this, administration of morphine along with a calcium channel blocker (CCB) can maintain a considerable lower level of intracellular calcium in the brain. Such a combination may also delay the development of dependence and lower the severity of withdrawal reactions. Previous studies have used different types L- and N-type CCBs such as nifedipine, verapamil, and diltiazem to study their effect on morphine dependence and withdrawal reactions.[10-12] Although these CCBs reduced the development of naloxone-precipitated withdrawal syndrome, their efficacy differs. Nimodipine, a CCB, is mainly used for the treatment of acute subarachnoid hemorrhage. It is highly selective for L-subtype calcium channels.[13] Since it is more lipophilic than other CCBs, it can cross the blood–brain barrier more easily.[14] It has minimal effect on blood pressure and is also neuroprotective in nature.[15] Therefore, the present study is an attempt to assess the effect of nimodipine, a highly selective L-subtype calcium channels blocker of dihydropyridine (DHP) group in morphine dependence and withdrawal reactions.

**Materials and Methods**

**Animals**

Male adult Wistar rats weighing 150–200 g were selected for the experiment. These rats were obtained from the Experimental Animal Facility of AIIMS New Delhi after prior approval by the Institutional Animal Ethics Committee. The animals were housed in a ventilated room (three animals/cage) with controlled temperature and artificial lighting conditions. Food and water was supplied *ad libitum*. A 12 h light/dark cycle was maintained. A total of 24 numbers of animals were used. These animals were randomly divided into various (four) experimental groups, each comprising six animals.

**Drugs**

The drugs used in the present study were morphine, nimodipine, naloxone, and normal saline (0.9% NaCl). The morphine was obtained from the government-approved pharmacy as ampules of morphine sulfate (15 mg/ml/ampoule). Naloxone and nimodipine were purchased in powder form from Sigma, USA. Naloxone was dissolved in normal saline. Nimodipine was dissolved in a vehicle containing physiological saline, polyethylene glycol 600, and absolute alcohol in a ratio of 2:2:1. The entire procedure was performed under dim light in a laminar flow in aseptic conditions. Normal saline was used as placebo in the present study. Physiological saline and morphine were injected subcutaneously (sc). Nimodipine (2 mg/kg) and naloxone (2 mg/kg) were used intraperitoneally (ip). Physiological saline was obtained from a local pharmacist.

**Induction of morphine dependence**

Morphine dependence was developed in rats by injecting constant doses of morphine, i.e., 10 mg/kg sc twice a day for 10 days.

**Development of withdrawal reactions and its scoring**

Naloxone 3 mg/kg ip was injected to morphine-dependent animals (MDAs) to precipitate withdrawal reactions. To observe the severity of withdrawal reactions in morphine-dependent rats, the rats were kept inside an open transparent acrylic glass container with a diameter of 23 cm and a height of 10 cm after administration of naloxone and observed for 30 min. The weight of the animals was determined before injection of naloxone and 2½ h after the injection of naloxone. To study the degree of withdrawal reactions, Gellert and Holtzman global withdrawal rating scale was used.[16] The scale consists of two groups of signs.

| Graded signs                      | Numerical values |
|-----------------------------------|------------------|
| Weight loss: 2½ h after           | 1                |
| Each 1% above the weight loss     | 1                |
| Number of escape attempts         | 2                |
| 1-2                               | 3                |
| >3                                | 4                |
| Number of wet-dog shakes          | 3                |
| 1-2                               | 4                |
| >3                                | 5                |
| Number of abdominal constrictions | 6                |
| Each one scored                   | 7                |
| Checked signs                     | 8                |
| Diarrheas                         | 9                |
| Teeth chattering                  | 10               |
| Swallowing movements              | 11               |
| Profuse salivations               | 12               |
| Chromodacryorrhea                 | 13               |
| Ptosis                            | 14               |
| Abnormal posture                  | 15               |
| Ejaculation                       | 16               |
| Irritability                      | 17               |

Numerical values indicate the weighing factor assigned to each feature and help in calculation of total withdrawal score.

**Experimental design**

Dependence was developed by injecting morphine 10 mg/kg for 10 days. Twenty-four animals were used in this experiment. The animals were divided into four groups (n = 6/group). Morphine was administered as mentioned above for 10 days in Groups 2, 3, and 4 to produce morphine dependence. Groups 3 and 4 received nimodipine in addition to morphine. Group 1: Saline treated control, Group 2: Only morphine as described above, Group 3: Morphine as per Group 2 with a single dose of nimodipine 2 mg/kg ip 20 min
before the administration of morphine once a day for 10 days, and Group 4: Morphine as per Group 2 followed by a single bolus dose of nimodipine 2 mg/kg ip only on the 11th day before the administration of naloxone. Naloxone 3 mg/kg ip was administered to all the groups on 11th day to precipitate the withdrawal reactions, and the withdrawal score was determined. The specific doses of morphine and nimodipine used in these experiments were selected based on both toxicity studies conducted in our laboratory as well as previous literature.[17]

### Statistical analysis

The data were entered in Microsoft Excel format and were analyzed using Systat version 700 and Epi Info version 6.04d. The statistical method used for comparison was Kruskal–Wallis one-way analysis of variance (ANOVA) and Mann–Whitney U-test. Student’s t-test and ANOVA were used to compare between groups wherever applicable. *P* < 0.05 was considered statistically significant.

### Results

**Group 1:** Saline treated control, **Group 2:** Only morphine as described above, **Group 3:** Morphine as per Group 2 with a single dose of nimodipine 2 mg/kg, ip 20 min before the administration of morphine once a day for 10 days, and **Group 4:** Morphine as per Group 2 followed by a single bolus dose of nimodipine 2 mg/kg ip only on the 11th day before the administration of naloxone [Table 1]. Naloxone 3 mg/kg ip was administered to all the groups. On 11th day to precipitate the withdrawal reactions, the withdrawal score was determined for the treated groups (mean ± standard error of the mean [SEM]).

The present study reflected the degree of morphine dependence developed by calculating the withdrawal score of morphine-dependent rats on injection of naloxone and the role of nimodipine in modifying/reducing these features of withdrawal reactions when it was administered along with morphine daily versus a single bolus dose administered before the precipitation of withdrawal reactions by injection of naloxone at the end of the experiment. The withdrawal score was determined for treated groups (mean ± SEM) and we consider *P* < 0.05 as statistically significant.

**Effect of daily administration of nimodipine along with morphine on morphine withdrawal**

Morphine dependence was developed in rats by administration of morphine at constant doses of 10 mg/kg/twice a day for 10 days. Rats developed significant morphine dependence in these 10 days as evidenced by development of morphine withdrawal on administration of naloxone at a dose of 3 mg/kg ip at the end of the experiment. Daily administration of nimodipine once a day 20 min before the administration of first dose of morphine of that day had no significant effect in morphine withdrawal as evidenced from total withdrawal score Group II versus Group III determined as a treated groups (mean ± SEM) and we consider *P* < 0.05 as significant.

### Effect of single bolus dose of nimodipine on morphine withdrawal

Total withdrawal score was significantly reduced when the comparison was made between morphine-dependent rats receiving a single bolus dose of nimodipine (2 mg/kg ip) 20 min before naloxone administration versus morphine-dependent rats receiving only naloxone at the end of the experiment (The dose of morphine was kept constant in both groups i.e., 10 mg/kg/twice a day for 10 days) Group II versus Group IV of the table. This was mainly due to reduction/control over diarrhea, weight loss, and irritability determined as treated groups (mean ± SEM) and we consider *P* < 0.05 as significant.

**Effect of nimodipine on individual features of morphine withdrawal**

Administration of nimodipine also modulated the individual parameters of morphine withdrawal. Individual features such as weight loss, wet-dog shakes, diarrhea, irritability, and ptosis were reduced significantly (*P* < 0.05).

### Discussion

Morphine administration (MA) may lead to morphine dependence. This dependence will manifest with unpleasant withdrawal reactions when there is sudden discontinuation of morphine or administration of morphine antagonists such as naloxone. The present study reflects

### Table 1: Results obtained with effect of nimodipine on morphine related to withdrawal syndrome

| Groups | Baseline | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
|--------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| I      | 3.2±0.2  | 3.1±0.1| 3.3±0.1| 3.6±0.1| 2.8±0.0| 3.4±0.2| 3.1±0.2| 2.8±0.1| 2.6±0.1| 2.3±0.2| 2.2±0.1|
| II     | 3.1±0.1  | 9.9±0.1| 8.9±0.4| 8.9±0.4| 5.7±0.9| 4.6±1.1| 4.8±0.8| 4.8±1.1| 3.5±0.4| 3.4±0.2| 3.5±0.5|
| III    | 2.81±0.1 | 10.0±0.1| 10.0±0.2| 9.9±0.1| 8.8±0.5| 8.2±0.8| 9.0±0.4| 7.7±0.8| 7.1±0.9| 5.9±1.1| 6.8±1.1|
| IV     | 2.81±0.1 | 10.0±0.2| 10.0±1  | 9.2±0.7| 9.0±0.5| 8.0±0.8| 8.0±0.9| 8.0±0.6| 6.3±0.9| 5.8±0.9| 4.9±0.6|

NB: On the eleventh day to precipitate the withdrawal reactions, the withdrawal score was determined for treated groups (mean±SEM), and we consider *P*<0.05 as significant. Morphine-related withdrawal syndrome, naloxone, nimodipine, pharmacotherapy. SEM: Standard error of mean
the preventive effect of a cerebroselective CCB nimodipine on morphine dependence and withdrawal reactions. Our study showed that a single bolus dose of nimodipine when administered before naloxone administration was more effective in controlling morphine withdrawal reactions than continuous administration of nimodipine along with morphine. The bolus dose reduced the severity of opioid withdrawal significantly in morphine-dependent rats. The important features of withdrawal reactions such as weight loss, diarrhea, irritability, and ptosis were significantly reduced by nimodipine when it was administered as a bolus dose before the administration of naloxone. Our results also showed that nimodipine when administered along with morphine had no significant effect on withdrawal reactions. Intracellular calcium concentration as well as L-subtype calcium channels plays an essential role in opioid dependence. Acute MA decreases the intracellular calcium concentration by closing the voltage-gated calcium channels, but on chronic administration, there is an increase in basal free intracellular calcium level.[4-6] This may be possibly due to upregulation of L-type calcium channels on chronic administration of morphine.[8,88] Administration of naloxone causes a sharp increase in intracellular calcium in various regions of the brain in morphine-dependent animals.[9] This leads to increased release of neurotransmitters and manifestations of withdrawal reactions. We investigate the effects of sinomenine on morphine withdrawal response and acetylcholine (Ach)-induced contracture in isolated guinea pig ileum. The withdrawal contracture was elicited by subjecting isolated ileum incubated with morphine (3 μmol/L) at 37.5°C for 4 h to naloxone (1 μmol/L) treatment. The sinomenine (10, 50, 250 μmol/L) and nimodipine (Nim, 0.1 μmol/L) were administered 1 min before and after naloxone in morphine-dependent ilea bathed in Krebs solution containing morphine. To observed the changes in the withdrawal contracture of the ileum. The effect of sinomenine (10, 50, 250 μmol/L) on the contracture of untreated ileum in Krebs solution elicited by Ach was also observed. Followed by this, naloxone-induced withdrawal contracture or Ach-induced contracture of the ileum was significantly decreased in a dose-dependent manner. It is indicating that sinomenine can inhibit morphine withdrawal symptoms in guinea pigs.

Evaluating the effects of tetrodrine (Tet) and nimodipine (Nim) on the morphine (Mor) withdrawal response in the isolated guinea pig ileum. The withdrawal contracture was elicited by addition of naloxone (Nal) (1 μmol/L) to the isolated naive ileum incubated with Mor (3 μmol/L) at 37.5°C for 4 h or to the ileum obtained from Mor-dependent guinea pig. When Nim (0.01, 0.05, and 0.1 μmol/L) or Tet (1, 10, and 50 μmol/L) was added 1 min before Nal in the naive ilea bathed in Krebs solution containing Mor, or when the ilea from Mor-dependent guinea pigs were incubated with Nim (0.01, 0.05, and 0.1 μmol/L) or Tet (1, 10, and 50 μmol/L) for 15 min, or when Nim (5 and 10 mg/kg, ip) or Tet (15 and 30 mg/kg, ip) was administered in vivo to Mor-dependent guinea pigs, the Nal-precipitated withdrawal contracture was significantly decreased in a dose-dependent manner. Tet and Nim, Ca2+ channel blockers, could inhibit the Nal-precipitated Mor withdrawal response in the isolated guinea pig ileum.[10] (1) The actions of the opioid agonists morphine and methionine-enkephalin (met-enkephalin) on the calcium channel currents (IBa) of acutely isolated locus coeruleus (LC) neurons from morphine-dependent and vehicle-treated rats were examined using whole cell patch clamp techniques. (2) In LC neurons maintained in 5 μM morphine, co-superfusion of naloxone (1 μM) or the mu-opioid receptor antagonist CTAP (D-Phe-Cys-Tyr-D-T rp-Arg-Thr-Pen-Thr-NH2 1 μM) with morphine resulted in a significant increase in the amplitude of IBa. The increases in IBa were not different in neurons from morphine-dependent or vehicle rats. The increase in IBa was mimicked by washing off morphine, but not by co-superfusion of the kappa receptor antagonist norbinaltorphimine (300 nM) or the delta receptor antagonist ICI-174864 (1 μM). (3) In spontaneously withdrawn LC neurons from morphine-dependent rats, met-enkephalin (pD2 7.1, maximum inhibition 49%) and morphine (pD2 6.5, maximum inhibition 33%), inhibited IBa in all cells. In cells from vehicle rats the pD2 for met-enkephalin was 7.3, maximum inhibition 52%, while the pD2 for morphine was 6.6 and the maximum inhibition 43% (P < 0.05 versus cells from morphine-dependent rats). (4) IBa in LC neurons was mostly comprised of omega-conotoxin GVIA-(N-type) and omega-agatoxin IVA-(P/Q-type) sensitive components, with lesser amounts of nimodipine-sensitive current and current resistant to all three blockers. Neither the density of IBa nor the proportion of any of the components of IBa differed between neurons from morphine-dependent or vehicle-treated rats. (5) This study demonstrates that in morphine-dependent rats, morphine and met-enkephalin modulation of somatic IBa in LC neurons display modest tolerance compared with untreated rats. Further, chronic morphine treatment does not alter the type or density of IBa in LC neurons. These results provide more evidence that functional mu-opioid receptor coupling is not dramatically altered in the LC in morphine-dependent rats.[11] Moreover, the development of dependence was assessed in the naloxone precipitation test after 13 days of morphine (20–30 mg/kg ip) administration. L-type Ca2+ channels were assayed in the cerebral cortex as [3H]nitrendipine-binding sites. Blood pressure was
monitored from the tail by a noninvasive method. Single-unit recordings in anesthetized animals revealed that the L-type calcium channel antagonist, nimodipine (10 mg/kg), attenuated the activation of LC neurons produced by naltrexone in opiate-dependent rats. Nimodipine also dose-dependently (1 mg/kg, 10 mg/kg, ip) inhibited the display of naloxone-precipitated withdrawal behaviors with a time course similar to that of nimodipine effects on the LC. These data suggest that nimodipine may suppress opiate withdrawal via noradrenergic mechanisms.[13] The effect of chronic administration of DHP calcium channel antagonist nimodipine (1 mg/kg/day) given concurrently with morphine on the signs of morphine withdrawal and on the [3H]nitrendipine binding in the rat brain has been investigated. Chronic MA in increasing daily doses from 20 mg/kg to 70 mg/kg for 24 days and consequent withdrawal for 24 h induced loss of body weight, wet-dog shakes, episodes of writhing and yawning behavior. The density of [3H]nitrendipine binding was elevated in the cortex and limbic structures but not in the striatum after chronic morphine treatment. Chronic concurrent administration of nimodipine prevented the loss of body weight and reduced the scores of wet-dog shakes and writhing but did not affect yawning behavior at 24 h after morphine withdrawal. These results suggest that chronic nimodipine treatment attenuates the development of the withdrawal signs which occur upon the termination of chronic morphine treatment by preventing the up-regulation of the central DHP-sensitive binding sites.[14] (1) Nimodipine, diltiazem, and BAY K 8644 decreased the incidence of wet-dog shakes, teeth chattering, grooming, and diarrhea to a similar degree of clonidine. (2) Nimodipine, diltiazem, and BAY K 8644 had no effect on changes in the serotonin metabolism induced by naloxone-precipitated abstinence syndrome. (3) Clonidine decreased the ratio of serotonin turnover in the brain of morphine-dependent rats. (4) From these experiments, it is concluded that nimodipine, diltiazem, and BAY K 8644 exert their effects in preventing morphine withdrawal symptoms through a mechanism independent of the serotonergic system.[15] (1) The effects of clonidine, nimodipine, and diltiazem, on the in vitro withdrawal contracture induced by naloxone in the guinea-pig ileum obtained from MDA's, were evaluated. (2) The in vitro incubation with clonidine (0.01, 0.1, and 1 μM), diltiazem (0.25, 0.1, and 1 μ) or nimodipine (0.05, 0.1, and 1 μ) reduced significantly the force of the contracture induced by naloxone in the morphine-dependent guinea-pig ileum. (3) The ip administration of clonidine (0.3 mg/kg), nimodipine (5 mg/kg), or diltiazem (20 mg/kg) reduced the contractile response induced by naloxone in the morphine-dependent guinea-pig ileum. (4) It is concluded that at least part of the effect of clonidine, nimodipine, and diltiazem on withdrawal contractures, it is mediated through a peripheral, rather than a central site of action. Even though the mechanism responsible for the effect of the CCBs differs from that of alpha 2-adrenoceptor agonists, all of the drugs tested prevented the contracture induced by naloxone in morphine-dependent guinea-pig ileum.[16] Regulation of L-type Ca2+ channels by morphine in rat brain was determined by the binding of [3H] nimodipine. Morphine, administered by sc pellet implantation, increased the density of [3H] nimodipine binding sites in a time- and dose-dependent manner and this effect was reversible upon removal of the pellets. Increases in these DHP sites were localized to the cortex, hippocampus, hypothalamus, and brainstem but not to the cerebellum and striatum. Additional experiments were performed to test the ability of different Ca2+ channel antagonists to affect naloxone-precipitated withdrawal in morphine-dependent mice and rats. These drugs effectively reduced the incidence of naloxone-induced jumping in mice and several of the withdrawal signs in rats. Taken together, our study underscores the plasticity of brain L-type Ca2+ channels and suggests that their upregulation might contribute to morphine dependence.[17] The effects of the Ca2+-channel blockers verapamil and nimodipine, on the behavioral signs of naloxone (1 mg/kg)-induced abstinence syndrome in morphine-dependent rats, were evaluated. The content of noradrenaline (NA) and of its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) was measured, using high-performance liquid chromatography and electrochemical detection or gas chromatography-mass spectrometry, in various brain regions of these animals. Possible interactions of nimodipine and verapamil with opioid receptors were evaluated by examining their ability to displace [3H] naloxone binding to brain membranes. Verapamil (5, 10, and 50 mg/kg) and nimodipine (1, 5, and 10 mg/kg) dose-dependently reduced most of the signs of morphine abstinence. Naloxone-precipitated abstinence decreased the NA content in the cortex, hippocampus, brainstem, and cerebellum. In the same brain regions, the content of MHPG increased, suggesting an increased release of the amine during morphine abstinence. Nimodipine (10 mg/kg intravenous) did not change the content of NA or MHPG in the cortex, hippocampus, and brainstem. However, nimodipine pre-treatment markedly reduced the changes in NA and MHPG content induced by the abstinence syndrome. Neither verapamil nor nimodipine displaced [3H] naloxone from its binding sites.[18] Administration of morphine for a longer period also results in upregulation of DHP binding sites in regions such as cerebral cortex, hippocampus, hypothalamus, and brainstem dorsal raphe nucleus of rat brain.[19] This
upregulation makes the channels more sensitive to DHP in morphine-treated rats. In this scenario, use of CCB of DHP group blocks the entry of calcium ion into the neurons more effectively than non-DHP CCBs. Hence, nimodipine, a CCB of DHP group when administered before naloxone administration, may prevent the naloxone-induced elevation of calcium ion in the neurons and indirectly decreases the release of neurotransmitters responsible for various adverse withdrawal reactions. This decrease may be significant when a single bolus dose of nimodipine was administered to the morphine-dependent rats before the administration of naloxone as shown in our study. However, nimodipine given together with morphine prevents the upregulation of DHP binding sites and decreases the expression of L-VSCC as reported earlier from our laboratory. This may be the reason why nimodipine administered together with morphine for a relatively longer period did not produce significant decrease in withdrawal reactions in our study.

Neurotransmitters such as NA and dopamine are mainly involved in opioid dependence and withdrawal reactions. The norepinephrinergic neurons of LC are the key neurons involved in the expression of somatic signs of opioid withdrawal. Nimodipine attenuates the features of opioid withdrawal by the regulating the release of norepinephrine and its metabolites in morphine-dependent rats. Single bolus dose of nimodipine may decrease the level of calcium ion within the neurons significantly that prevent the release of norepinephrine. However, this effect is less marked when nimodipine is administered together with morphine for a longer period. Morphine dependence also influences neostriatal activity. Chronic opioid use decreases the level of dopamine and its metabolite in specific brain regions involved in morphine withdrawal. The level further decreases during opioid withdrawal. This may be responsible for development of features such as swallowing movements, plosis, and facial fasciculation observed during morphine withdrawal. Nimodipine attenuated these abnormal features possibly by normalizing the level of dopamine. CCBs may also reduce the severity of morphine withdrawal by other mechanisms such as decreasing the corticosterone secretions. Nimodipine also has potential for alleviating morphine addiction by selectively decreasing its incentive motivational property. Hence, the use of nimodipine along with morphine may also be advantageous in preventing dependence in clinical conditions necessitating chronic use of morphine.

**Current state of knowledge and future direction**

Although positive and negative reinforcement as a key components is present in many types of drug addiction still continued usage of drug stems from positive reinforcement of drug taking and negative reinforcement results from withdrawal along with quitting drugs. Therefore mesocorticolumbic dopamine mechanism originates in the ventral tegmental region and projects to terminal regions such as prefrontal cortex, amygdala, and acumen, is an important neural network wherein drug-induced neuroadaptations occurred in both types of reinforcement. Reinforcing influences of substance abuse contribute to increased dopaminergic neurotransmission in the Acb. Animal’s lever-press to keep increased DA rates over cocaine self-administration declined accumual DA rates are associated with morphine withdrawal. Hence, drug abstinence gives rise to physical withdrawal psychological withdrawal components are mediated through distinct neural systems. Opiate antagonists in the LC and the periaqueductal gray precipitated robust somatic withdrawal syndromes in MDAs. The Acb generates a few somatic symptoms exclusively MDAs. Direct administration of opiate antagonists into the Acb and amygdala brings about psychological withdrawal as indicated by the decline of lever pressing for food and conditioned place aversion. In addition, direct administration of opioid antagonists in the amygdala of MDAs was shown to contribute to moderate physical withdrawal. In addition, systemic DA agonist administration lowers both conditioned place aversions and physical withdrawal symptoms in MDAs that were treated with naloxone. It increasing phosphorylation of GluR1 and Acb indirectly implicates the Acb in both withdrawal components.

Morphine chronic misuse leads to opiate addiction range of symptoms occur after stopping or dramatically reducing morphine subsequent to the heavy and prolonged use tempting to suggested that neural mechanisms serve key roles in morphine withdrawal are quite important. We also directed that future association in cortisol, serotonin, melatonin, and beta-endorphin and other hormones unlike stress and antistress protein may also have major role associated with morphine withdrawal need to clarify in further research on different magnitude of this related study need to further study to clarify all context of view in neurobiochemistry.

**Conclusion**

The results of the present study indicate a crucial role of nimodipine in attenuating the severity of morphine withdrawal, which is often encountered during the pharmacotherapy with morphine or management of opioid dependence. Furthermore, in our study, a single bolus dose of nimodipine significantly reduced withdrawal reactions, which makes it an attractive
candidate for use in morphine-dependent patients. The use of single bolus dose will also lower the risk of adverse events associated with long-term CCB therapy. Hence, we strongly reported and give evidence that it add additional information in our human subject as well memory impairment, cell signaling, and phosphorylation of neuron.

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Conflicts of interest
There are no conflicts of interest.

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