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PROTECTIVE EFFECT OF THE CALCIUM CHANNEL BLOCKER, NIFEDIPINE, AGAINST ALCOHOL WITHDRAWAL-INDUCED ANXIETY IN RATS

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ABSTRACT

The objective of the present study was to measure anxiety as a sign of alcohol withdrawal in an animal model of anxiety and to assess the protective effect of the calcium channel blocker, nifedipine, against alcohol withdrawal-induced anxiety in male rats. Fifty-six adult male albino rats were used in the current study. Animals were randomly divided into two groups, normal rats and alcohol-exposed rats. The normal animals were given free access to tap water; the normal group stayed alcohol naïve for the remainder period of the study. The animals of the alcohol-exposed group were given free access to ethanol solution (10% v/v) in their home cages. Rats were given access to ethanol in their home cages for three cycles of 5 consecutive days, interspersed by two days of tap water. Each group, normal or alcohol-exposed group, was further randomly subdivided into 4 subgroups, 7 rats each; the animals of these subgroups received the following treatments: Normal saline (NaCl 0.9%), Diazepam at a dose of 0.5mg/kg, i.p. 30-minutes before anxiety test, Nifedipine at doses of 10mg/kg, i.p. 30-minutes before anxiety test, and Nifedipine, 5mg/kg, i.p. daily for 5 days a week for 3 weeks along with ethanol exposure. Test of anxiety was conducted after 3 cycles of tap water or ethanol exposure. Tap water or ethanol solution was removed from all animal cages at 4:00p.m the day before anxiety testing to induce water-deprivation in normal animals and alcohol-withdrawal in alcohol-exposed rats, respectively. The present results show that alcohol exposure was effective in producing anxiety as a sign of alcohol-withdrawal in rats. Treatment with the lower dose of nifedipine (5mg/kg), for three weeks along with alcohol exposure, was more effective in alleviating the alcohol-withdrawal-induced anxiety as compared to the acute effect of the higher dose (nifedipine, 10mg/kg). Diazepam induced a comparable anxiolytic effect. It could be concluded that calcium channel blockers may offer a possible substitute for benzodiazepine anxiolytics to treat alcohol-withdrawal symptoms because they do not induce physical dependence

INTRODUCTION

It is well established that chronic ethanol administration leads to tolerance and dependence[1]. Similar to other drugs of abuse, dependence to ethanol has been mainly demonstrated through the expression of a withdrawal syndrome[2]. Abrupt cessation from chronic ethanol administration induces a withdrawal syndrome characterized among other symptoms, by tremors, agitation, general rigidity, spontaneous seizures, and increased sensitivity to audiogenic as well as handling-induced seizures[3]. In addition to the somatic signs of withdrawal, the interruption from persistent intake with most drugs of abuse, including ethanol, can promote disturbances in affective states and emotionality, in particular, high levels of anxiety[4].

High-alcohol-drinking rats exhibited an excessive fear reaction in standard fear conditioning tasks[5]. Exposure to multiple withdrawals from moderate amounts of alcohol induces sensitization of anxiety-like behavior[6]. All these findings support the contention that prior experience with ethanol withdrawal facilitates the occurrence of exaggerated negative emotional responses, mainly fear and "anxiety", to environmental challenges.

Anxiolytics are among other agents used to treat withdrawal symptoms of drugs. Benzodiazepines are the most commonly prescribed anxiolytic drugs, being effective against a wide spectrum of anxiety disorders. However, addiction, tolerance, and dependence/ withdrawal may develop with these drugs, as well as adverse side effects that include sedation, cognitive and psychomotor impairments, and anterograde amnesia. Benzodiazepine withdrawal in human is associated with increased anxiety, insomnia, sensory disturbances, and seizures[7]. Similar symptoms have been observed in animals withdrawn from chronic benzodiazepine treatment[8]. Therefore, availability of a non-addicting alternative to substitute for benzodiazepines will be advantageous; calcium channel antagonists have been proposed as an alternative.

Calcium antagonists have been reported to be effective in the treatment of cardiac arrhythmias and other cardiovascular pathologies[9]. The calcium antagonists are chemically heterogeneous drugs that inhibit the uptake of calcium into cells through L-voltage-dependent channels. Calcium channels appear to exist in all neurons and provide a significant proportion of the activator calcium required for transmitter release. Evidence exists that calcium antagonists can have effects on neuronal function[10].

Nimodipine[11] and nifedipine[12] are able to pass the blood-brain barrier. Several reports suggest that they are also effective in the treatment of affective disorders such as mania[13], depression[14], behavioral changes induced by acute or chronic opioid treatments[15], convulsions[16], and withdrawal symptoms and seizures in alcohol-dependent rats[17].

The objective of current study was to measure anxiety as a sign of alcohol withdrawal in an objective animal model of anxiety and to assess the protective effect of calcium channel blocker, nifedipine against alcohol withdrawal-induced anxiety in male rats.

MATERIAL AND METHODS

Animals:

Fifty-six adult male albino rats weighing 120-150gm, obtained from the Egyptian Organization for Biological Products and Vaccines (Vacsera, Cairo, Egypt), were used in the current study. Animals were kept at 25-27° C on a 12-hours light-dark cycle in a room with relative humidity 35-70%. The animals were individually housed in stainless steel cages (40 X 30 X 17cm) with mesh floor, and hardwood bedding was used. Food and water were allowed ad libitum for an accommodation period of one week. Food was available throughout the study.

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Chemicals and experimental procedures:

Alcohol exposure:

After one week of habituation to the animal room, animals were randomly divided into two groups, normal rats and alcohol-exposed rats. The normal animals were given free access to tap water; the normal group stayed alcohol free for the remainder period of the study. The animals of the alcohol-exposed group were given free access to ethanol solution (10% v/v) in their home cages[18]. The alcohol solution is made up from ethyl alcohol (95%) diluted with tap water (Ethanol, 95%, ADWIC, El-Nasr Pharmaceutical Chemicals, Egypt). Rats were given access to ethanol in their home cages for three cycles of 5 consecutive days, interspersed by two days of tap water[19]. Spillage and evaporation were minimized by the use of bottle caps with ball bearings. With this procedure, the alcohol concentration stayed constant[20].

Study design:

Normal and alcohol-exposed group were further randomly subdivided into 4 subgroups, 7 rats each; the animals of these subgroups received the following treatments:

1. **Normal saline:** NaCl 0.9%, ADWIC, El-Nasr Pharmaceutical Chemicals, Egypt.
2. **Diazepam:** Valpam® ampoule, Amoun Co., Egypt; with a concentration of 5mg/1ml/ampoule), was diluted in saline (0.9%NaCl) and used at a dose of 0.5mg/kg, i.p, 30-minutes before anxiety test.
3. **Nifedipine (Eipico Co., Egypt):** Nifedipine powder was dissolved in cold normal saline with the addition of 2 drops of Tween-80 and used at doses of 10mg/kg, i.p, 30-minutes before anxiety test.
4. **Nifedipine**, 5mg/kg, i.p, daily for 5 days a week for 3 weeks along with ethanol exposure.

Test of anxiety was conducted after 3 cycles of tap water or ethanol exposure. Tap water or ethanol solution was removed from all animal cages at 4:00p.m the day before anxiety testing to induce water-deprivation in normal animals and alcohol-withdrawal in alcohol-exposed rats, respectively.

Table (1): A summary of the study design

Group	Treatment Subgroup (n=7)
Normal Group	Normal saline (0.9%NaCl, i.p. 30-min before anxiety test).
	Diazepam (0.5mg/kg, i.p. 30-min before anxiety test).
	Nifedipine (10mg/kg, i.p. 30-min before anxiety test).
	Nifedipine (5mg/kg, i.p, daily, 5 times a week a total of 15 injections).
Alcohol-Exposed Group	Normal saline (0.9%NaCl, i.p. 30-min before anxiety test).
	Diazepam (0.5mg/kg, i.p. 30-min before anxiety test).
	Nifedipine (10mg/kg, i.p. 30-min before anxiety test).
	Nifedipine (5mg/kg, i.p, daily, 5 times a week a total of 15 injections).

The Vogel conflict test of anxiety:

Vogel et al.[21] developed a conflict procedure, the Vogel conflict test, in which male rats were water-deprived for 48 hours and, during a test session of 3 min, drinking was punished by a mild but aversive shock delivered via the spout of the bottle every 20 licks. Accordingly, a specific, drug-induced increase in the number of shocks taken (equivalent to water drunk) was considered to reflect anxiolytic properties of a drug treatment. It is advantageous that both increases and decreases in anxiety can be revealed.

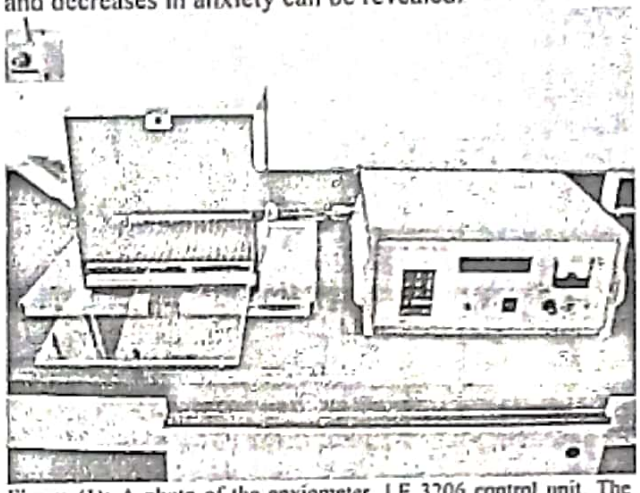

Figure (1): A photo of the anxiometer, LE 3206 control unit. The anxiometer is composed of a testing chamber and a shock generator device

Experimental procedures for anxiety test:

The shock parameters were set, shock length was 1 second, and shock intensity was 0.3 mAmp. The licks/shock ratio (LSR) was fixed at 20-licks/1-shock; the total trial length (TTL) time was 5 minutes. A shock was supplied each time the LSR is completed; the TTL time started only after the first completion of the LSR. The trial run until the TTL time was completed.

Statistical analysis:

Data were computed as the mean ± standard error of the mean and compared using SPSS (version 9). Multiple comparisons were carried out using one way analysis of variance (ANOVA) followed by Bonferroni test for selected pairs. For all comparisons, differences were considered significant at $p<0.05$.

RESULTS

The present results showed that alcohol exposure in the home cage for three cycles of 5 consecutive days interspersed by two days of tap water was effective in producing anxiety as a sign of alcohol-withdrawal. It was evident that alcohol withdrawal has induced anxiety in rats. Figure 2 shows that alcohol withdrawn-rats received a significantly lower number of shocks as compared to the normal, alcohol-naïve rats, which reflects a state of anxiety (Normal rats, 20±3.2 vs. Alcohol-withdrawal, 9±2.8, $p<0.05$, figure 2).

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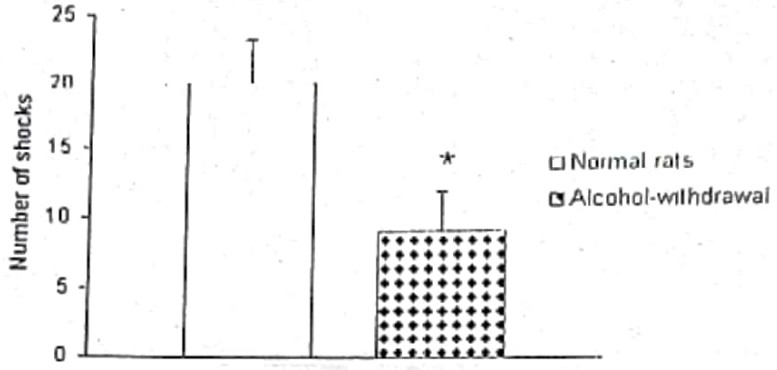

Figure (2): The number of shocks received by normal rats, in 5-min anxiety test, and by rats exposed to ethanol (10% v/v), on 5 consecutive days a week, for 3 weeks

*Significantly different from control animals at $p<0.5$

ANOVA analysis indicated a significant effect of drug treatment on the anxiety levels of rats ($p<0.05$). The traditional anxiolytic agent, diazepam, could increase the number of shocks taken by these animals. In the alcohol naïve group, diazepam treatment (0.5mg/kg) could significantly increase their number of shocks (Normal saline, 20±3.2 vs. Diazepam, 33±2.1, $p<0.05$, figure 3). Similarly, the higher dose of nifedipine (10mg/kg) produced a significant increase in the number of shocks received by the alcohol naïve animals (29±1.8, $p<0.05$, figure 3). In contrast, the lower dose of nifedipine (5mg/kg) failed to demonstrate a comparable effect in the alcohol naïve animals (figure 3).

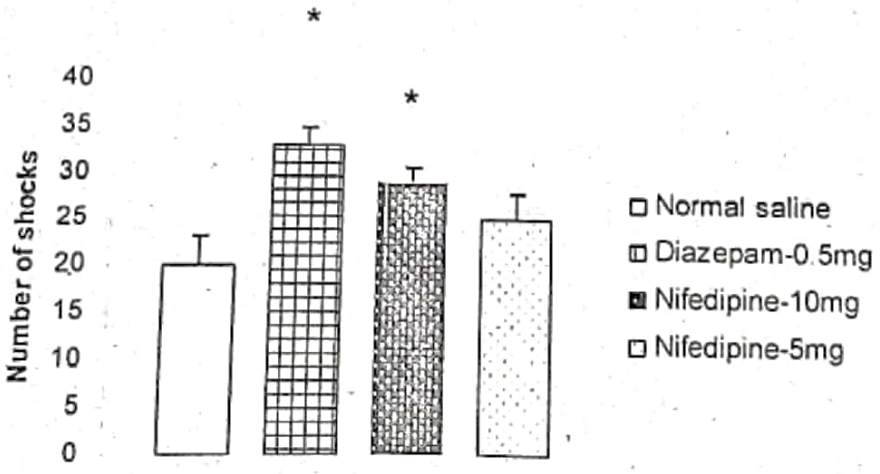

Figure (3): Effects of diazepam (0.5 mg/kg, i.p), nifedipine (10mg/kg, i.p) and nifedipine (5mg/kg, i.p. daily for 5 consecutive days a week for 3 weeks) on the number of shocks received by normal rats in 5-min anxiety test

*Significantly different from control animals at $p<0.5$

Again, various drug treatments exhibited a significant effect on the number of shocks taken by alcohol-withdrawn rats (ANOVA, $p<0.05$). Diazepam treatment (0.5mg/kg) produced a significant elevation in the number of shocks taken by the alcohol-withdrawn rats (Alcohol withdrawal, 9±2.8 vs. Diazepam, 31±2.7, $p<0.05$, figure 4). Parallel to this effect of diazepam, the high dose of nifedipine (10mg/kg) produced a significant increase in the number of shocks taken by the alcohol-withdrawn animals (19±2.1, $p<0.05$, figure 4).

Interestingly, unlike the effect on the alcohol naïve rats, nifedipine (5mg/kg) produced a significant increase in the number of shocks received by the alcohol-withdrawn animals (28±2.4, $p<0.05$, figure 4). This effect was also significantly higher than the acute effect of the higher dose of nifedipine (Nifedipine-10mg, 19±2.1 vs. Nifedipine-5mg, 28±2.4, $p<0.05$, figure 4).

Thus, the chronic administration of the lower dose of nifedipine (5mg/kg) along with alcohol exposure was more effective in alleviating the alcohol-withdrawal-induced anxiety as compared to the acute effect of the higher dose (nifedipine, 10mg/kg).

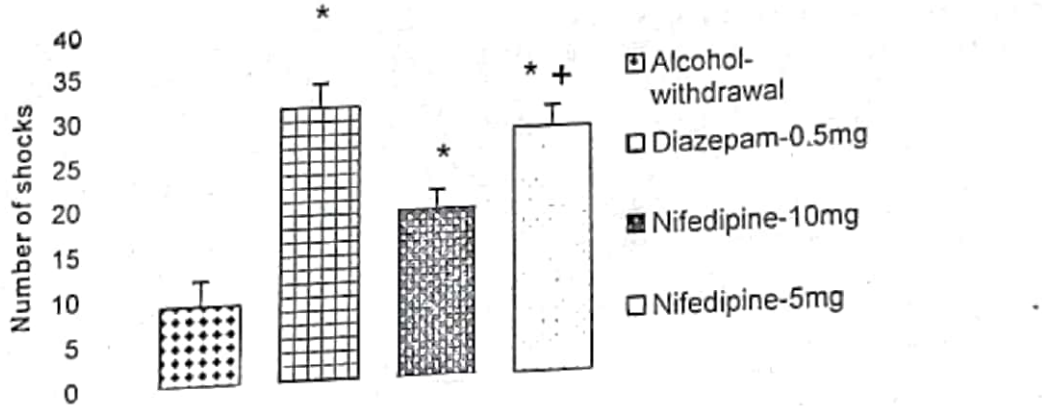

Figure (4): Effects of diazepam (0.5 mg/kg, i.p), nifedipine (10mg/kg, i.p) and nifedipine (5mg/kg, i.p, daily for 5 consecutive days a week for 3 weeks) on the number of shocks received by alcohol-withdrawn rats in 5-min anxiety test

*Significantly different from control animals at $p<0.5$

+Nifedipine-5mg is significantly different from Nifedipine-10mg/kg at $p<0.5$

DISCUSSION

The current results demonstrated a significant effect of calcium channel blocker, nifedipine, at two dose levels on the alcohol withdrawal-induced anxiety; this may reflect an important role of cacium channels in alcohol withdrawal syndrome in rats. Previous research showed that among various neurochemical events supposed to participate in the development of ethanol physical dependence, one is enhanced function of L-type high voltage-gated calcium channel as reported in animal brains after long-term treatment with ethanol[22], and this alteration has been considered to be due to increased numbers of binding sites of dihydropyridines, antagonists selective to L-type high voltage-gated calcium channel[23]. Similar alterations in the bindings of radiolabled dihydropyredines have also been reported in the brain of animals with ethanol physical dependence[24]. These findings suggest that up-regulation of L-type high voltage-gated calcium channels in the brain is a neurochemical change showing the establishment of ethanol physical dependence, and may participate in producing neurological and psychological signs observed in alcoholics and animals dependent on alcohol.

Increasing evidence suggests that changes in calcium channel function play an essential role in opioid tolerance and dependence development. Biochemical data indicate that important alterations in neural calcium disposition can occur during the chronic administration of opioids, so that acute morphine reduces synaptosomal calcium, but, as dependence develops, calcium levels in synaptosomes increase proportionately[25]. In addition, L-type calcium channels are up-regulated in rats showing signs of morphine withdrawal[26]. An increased calcium influx through up-regulated L-type calcium channels may be involved in the development of opioid dependence. In this situation, the elevation of intracellular calcium enhances the release of neurotransmitters that are involved in the induction of opioid withdrawal[27]. Calcium channel antagonists have been shown to attenuate the signs of physical dependence in animals[28]. Additionally, concomitant administration of calcium channel antagonists with morphine completely prevents the naloxone-induced up-regulation of [3H] nitrendipine binding sites[29]. This suggests that morphine administered during L-type calcium channel blockade is unable to induce changes related to some neurochemical effects underlying the abstinence syndrome.

In agreement with the present anxiolytic effect of nifedipine, calcium channel blockers have been

shown to have some efficacy in the treatment of mood disorders[30]. In animal studies, the administration of calcium channel blockers has been reported to exert an anxiolytic-like effect[31] and in the paradigm consisting of measuring water intake by animals placed in a novel anxiogenic environment[32]. Calcium channel blockers produced a reduction in anxiogenic-like behavior normally associated with withdrawal syndrome after chronic exposure to drugs of abuse[33]. The calcium channel blockers dihydropyridines have selective effects in decreasing alcohol withdrawal hyperexcitability without affecting alcohol pharmacokinetics[34].

The current results showed that repeated administration of the calcium channel blocker, nifedipine-5mg, along with alcohol exposure could effectively block the alcohol withdrawal-induced anxiety; this effect was absent in the alcohol naïve rats; this may reflect an important role of calcium channels up regulation during drug dependence. In line with this concept, El Ganouni et al.[35] showed that withdrawal from chronic diazepam was followed by a decrease in water intake in a novel environment, suggesting increased anxiety. This rebound of anxiety could be blocked by nifedipine at 10 mg/kg, like that achieved by a further administration of diazepam. Moreover, nifedipine, at the daily dose of 5mg/kg, administered concomitantly with diazepam, also provided good protection against the withdrawal syndrome. Indeed, repeated administration of nifedipine at 5mg/kg/day for 8 days was more effective than acute treatment. These observations are in line with previous data showing an anti-withdrawal-like effect of calcium channel blockers after morphine withdrawal[36].

Several mechanisms for an anxiolytic effect of the calcium channel blockers have been suggested. It has previously been shown that the anxiolytic-like effect of nifedipine could be modulated by 5-HT_{1A} receptor antagonist, ipsapirone[37]. Thus, some influence of calcium channel blockers on 5-HT neurotransmission might be implicated in the anxiolytic properties of these drugs. It is well established that, through the stimulation of 5-HT_{1A} and 5-HT_{2A} receptors, an increased output of 5-HT can enhance corticosterone secretion[38]; corticosterone enters the brain, where it binds to intracellular receptors that are abundant in limbic areas. In these areas, corticosterone can increase the amplitude of sustained high-voltage activated calcium currents (L-type, at which dihydropyridine calcium channel blockers act) and enhance calcium influx in neurons within the limbic system[39]. Such actions could modify the activity of limbic pathways that are implicated in anxiety behavior and regulated by 5-HT neurotransmission.

Moreover, the inhibition of dopaminergic activity has been considered a neurological correlate of the dysphoric and depressive symptoms of ethanol abstinence[40]. Accordingly, associated with the inhibition of the mesolimbic dopamine system during withdrawal is a rebound depression of reward mechanism[41]. Thus, the alterations of dopamine neurotransmission by chronic ethanol may be relevant to the mechanisms of ethanol dependence. L type calcium channels appear to be involved in the effects of ethanol on the dopaminergic system as dihydropridine calcium antagonists abolish the stimulatory effect of ethanol on dopamine release[42]. The mechanism by which dopamine neurotransmission is inhibited after withdrawal from chronic intoxication is recommended for further research[43].

In conclusion, a brief word of caution should be mentioned that calcium channel blockers might offer a possible substitute for benzodiazepine anxiolytics to treat alcohol withdrawal symptoms because they do not induce physical dependence. It can be proposed that calcium channel blockers may be useful to reduce and/or prevent anxiety caused by abrupt cessation of alcohol consumption and possibly also to other drugs producing dependence. Moreover, some somatic consequences of drug withdrawal, such as tachycardia and other cardiac disorders, would be successfully treated at the same time, given the known cardiac effects of dihydropyridines. Relevant clinical trials should be performed to assess the possible human application of such data.

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التأثير الواقي لغالق قنوات الكالسيوم (نيفديبين) ضد القلق المحدث بسحب الكحول فى الجرذان

السيد العوضى

قسم الأدوية والسموم- كلية الصيدلة- جامعة قناة السويس- الإسماعيلية- مصر

تهدف الدراسة الحالية لقياس القلق ، كعرض لسحب الكحول ، ودراسة التأثير الواقي لغالقات قنوات الكالسيوم (نيفديبين) ضد القلق المحدث بسحب الكحول فى ذكور الجرذان .

أجريت الدراسة على ستة وخمسين من جرذان التجارب الذكور ، قسمت إلى مجموعتين ، مجموعة حيوانات طبيعية ومجموعة معرضة للكحول ، تعرضت المجموعة الأخيرة لمحلول الكحول 10% حجم/حجم فى أقفاص الحيوانات يوميا لمدة 5 أيام أسبوعيا على مدي 3 أسابيع ، عولجت المجموعات بالحقن بمحلول ملح 0.9% او ديازيبام 0.5 مجم/كجم بالتجويف البريتونى قبل اختبار القلق بنصف ساعة او نيفديبين 10 مجم/كجم بالتجويف البريتونى قبل اختبار القلق بنصف ساعة أو نيفديبين 5 مجم /كجم بالتجويف البريتونى يوميا لمدة 5 أيام أسبوعيا على مدى 3 أسابيع بعدها أجريت اختبارات القلق.

أظهرت النتائج ان سحب الكحول تسبب فى حدوث القلق عند الجرذان ، وان علاج الجرذان بمادة النيفديبين قد اظهر فعالية عالية فى علاج القلق خاصة الجرعة اليومية من النيفديبين (5 مجم/كجم) مقارنة بتأثير الديازيبام على القلق.

تخلص الدراسة إلى أن غالقات الكالسيوم الخالية من خواص الإدمان قد تمثل بديلا لمادة الديازيبام لعلاج أعراض سحب الكحول ويكون تطبيق ذلك بعد عمل الدراسات الإكلينيكية اللازمة.