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# Investigation of the impact of antiparasitic drug moxidectin on the rewarding effects of alcohol

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**Abstract.** Alcohol addiction or alcoholism constitutes a significant risk factor worldwide for morbidity and mortality. Moxidectin is a recently approved anthelmintic drug, which also activates the gamma-aminobutyric acid receptors. The objective of the present study was to examine the impact of moxidectin on rewarding effects of ethanol in the conditioned place preference (CPP) model in mice. In separate experiments, mice were administered intraperitoneal (i.p.) injections of moxidectin (5 or 10 mg/kg) before a) acquisition of alcohol-induced CPP, b) each extinction session, and c) alcohol-induced reinstatement of CPP. The present experiments provide consistent data about ethanol place preference in mice (2 g/kg, i.p.), with mice in all tests spending significantly more time on the ethanol-paired side. The acquisition of the CPP response to ethanol was prevented by the administration of moxidectin at a dose of 10 mg/kg. Additionally, moxidectin treatment accelerated the extinction of ethanol CPP when given repeatedly during the extinction phase. Ethanol-induced reinstatement of CPP following an extinction phase was inhibited by moxidectin. Ethanol alone and co-administration with moxidectin did not change locomotor activity and motor coordination. In conclusion, we suggest that moxidectin may be a promising therapeutic candidate for prevention of ethanol-induced addiction and relapse as well as detoxification.

**Key words:** Alcohol addiction — Moxidectin — Conditioned place preference — Mice

## Introduction

Drug and substance misuse involves the excessive use of drugs and substances that tend to stimulate brain reward mechanisms and reinforce behaviors and the creation of memories. Alcohol abuse is a chronic relapsing and complex brain disorder characterized by three distinct stages in the addiction period that lead to neuroadaptive changes in the brain (Huynh et al. 2019; Bota et al. 2021). The damaging effects of frequent alcohol consumption on various organs and systems were repeatedly illustrated (Witkiewitz et al. 2020; Bota et al. 2021). Alcohol consumption increases the risk of disorders of the stomach, esophagus, intestines, pancreas, and liver by inducing carcinogenesis and influencing inflammatory processes. Addiction can lead to heart failure, hypertension, arrhythmia, and alcoholic cardiomyopathy, and also hematopoietic diseases (Batra et al. 2016; Carvalho et al. 2019). Fetal alcohol spectrum disorder is estimated to occur in about 630,000 children born every year globally (Lange et al. 2017; Bukiya and Dopico 2018; Tung et al. 2020).

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An estimated 3 million people in the world die due to alcohol use. Furthermore, alcohol abuse is indicated to be one of the leading causes of death among people aged between 15 and 49 years (Pan et al. 2018).

Moxidectin was developed as an alternative therapy to ivermectin, an antiparasitic drug for use in humans. It is a potent, broad-spectrum endectocide with action against a wide range of nematodes, acari, and insects. To date, no significant moxidectin-related clinical malformations were demonstrated (Huynh et al. 2017, 2019). With moxidectin being approved for human use, it could represent another macrocyclic lactone candidate that could be used as pharmacotherapy for alcohol dependence (Huynh et al. 2017, 2019). Gamma-aminobutyric acid (GABA) receptors have an important role in alcohol addiction (Hillmer et al. 2016; Khoja et al. 2018). Among effective drugs useful in the treatment of alcohol addiction, GABAB-ergic medications were tested with reassuring results (i.e. sodium oxybate, baclofen, gabapentin, pregabalin and tiagabine) (Mirijello et al. 2015; Minozzi et al. 2018; Pan et al. 2018; Witkiewitz et al. 2019). Moxidectin, although it shows activity potentiating GABAA receptors, the degree of GABAA potentiation is significantly less than ivermectin's activity on GABAA receptors (Huynh et al. 2017). Several recent reports in the literature suggest moxidectin offers lower neurotoxicity potential compared to ivermectin (Huynh et al. 2017). The more advantageous central nervous system safety profile of moxidectin is thought to be due to: 1) moxidectin having lower activity on GABA receptors compared to ivermectin which should be an advantage with respect to decreasing contraindications, and 2) differential transport across the blood-brain barrier, with moxidectin being a more limited substrate for P-glycoprotein transporter and lesser dependence on P-glycoprotein for removal from the central nervous system (Huynh et al. 2017). Prior research suggests that moxidectin activates P2X4 and GABA receptors (Ménez et al. 2012; Franklin et al. 2014; Huynh et al. 2017; Spampanato et al. 2018). P2X4 and GABA receptor systems are observed to be crucial in alcohol addiction (Franklin et al. 2014; Huynh et al. 2017).

The conditioned place preference (CPP) model is based on the principle of classical (Pavlovian) conditioning and is generally applied to measure the rewarding effect of addictive substances and drugs, such as ethanol (Napier et al. 2013; Hilderbrand and Lasek 2014; McKendrick and Graziane 2020; Yunusoğlu 2021a). In the paradigm, two different environments are paired separately with treatment by placebo or drug. During subsequent numerous pairing (conditioning phase) sessions, animals (rodents or monkeys) are given the opportunity to explore both environments (McKendrick and Graziane 2020; Yunusoğlu 2022b). With administration of substances (alcohol) and drugs known to produce pleasurable effects in humans, animals (i.e. mice or rats) tend to develop a preference for (spend more time) the environment paired with that drug or substance (Napier et al. 2013; McKendrick and Graziane 2020).

Comprehensive interventions are necessary to decrease mortality and disability among patients with alcohol addiction (Carvalho et al. 2019; Yunusoğlu 2022b). The present treatment approaches commonly used for chronic alcoholism include psychotherapy (often behavior therapy) and pharmacotherapy (i.e. disulfiram, naltrexone, and acamprosate) (Carvalho et al. 2019). Currently, there is no fully effective drug available for the treatment of alcohol addiction. Hence, there is a requirement to develop innovative medications with low side effect profiles for the treatment of alcohol addiction.

The purpose of the present study was to investigate the effect of moxidectin on the acquisition, extinction, and reinstatement of ethanol-induced CPP in mice.

## Material and Methods

### *Animals and housing*

Intact adult male Swiss albino mice (22–24 g at the start of the experiment) were subjects for the study. They were housed 4–5 *per* cage in a 22–23°C humidity-controlled facility. Food and water were available *ad libitum*. The mice were acclimatized for 4–5 days before the experiment. Experiments were performed during the light cycle and were approved by Animal Experiments at Van Yüzüncü Yıl University. Efforts were made to decrease the number of mice employed ($n$ = 6–8/group). Measures that lessened distress to the mice were implemented.

### *Chemicals*

Moxidectin (C37H53NO8) and ethanol were purchased from Sigma (Louis, USA). The doses of moxidectin and alcohol were determined based on previous research (Huynh et al. 2017; McKendrick and Graziane 2020). Ethanol (20% v/v) was diluted in 0.9% sodium chloride (NaCl) solution and administered intraperitoneally (i.p.) at a dose of 2 g/kg. Moxidectin was dissolved in a mixture of polyethylene glycol 200 and 0.9% NaCl solution, and was administered i.p. with a volume of 10 ml/kg.

### *Apparatus*

The CPP test was conducted in an apparatus consisting of two equal-sized chambers (20×20×20 cm) that can be separated by guillotine doors. Each chamber had a different wall design and flooring; one chamber had a white wall and stainless-steel rod floor and the other chamber had a striped white and black wall with a stainless-steel mesh floor (McKendrick and Graziane 2020).

*Habituation phase*

Animals were located in the center of the CPP device with the doors open with free access to both chambers for 15 min.

*Pre-conditioning test*

In order to confirm if mice displayed a normal preference for either of the chambers, mice were located in the center of the CPP device with the doors open and allowed spontaneous entry to both chambers for 15 min. No injection was given on the day of the pre-conditioning test. An unbiased design was applied because mice displayed no preference for either of the chambers in the pre-conditioning test. The time spent by the mice in each chamber was recorded during 15 min.

*Conditioning phase*

Through the conditioning phase, mice were injected with ethanol (2 g/kg, i.p.; day 2, 4, 6, 8) or saline (12.5 ml/kg, i.p.; day 3, 5, 7, 9) on alternate days. Following injections, the mice were enclosed in the assigned drug-paired chamber or saline-paired chamber for 30 min. This test was performed in counterbalanced manner. The control group received saline and was conditioned (12.5 ml/kg, i.p.).

*Effects of moxidectin on the acquisition of ethanol-induced CPP*

Animals were divided into four groups: the control group (saline + saline); alcohol group (saline + alcohol) and moxidectin treatment groups (moxidectin (5 and 10 mg/kg, i.p.) + alcohol respectively). Through the conditioning phase, 10 and 20 mg/kg moxidectin doses were administered i.p. 30 min before the alcohol injections.

*Moxidectin effects on extinction of ethanol-induced CPP*

Extinction of ethanol-linked CPP was observed after the creation of CPP. Throughout this period, the animals were separated similarly into four groups given moxidectin (5 and 10 mg/kg, i.p.) or saline 30 min prior to the daily extinction test. Throughout all tests, the mice were located in the CPP device with spontaneous entry to both chambers for 15 min. The time spent by the mice in each chamber was recorded. A graphical timeline for each experiment is shown in Figure 1.

*Moxidectin effects on drug-priming reinstatement of ethanol-induced CPP*

Extinction of ethanol-linked CPP was restored with priming administration of ethanol at a dose of 0.4 g/kg, i.p. One day after the last extinction test, mice given moxidectin (5 and 10 mg/kg, i.p.), or saline 30 min prior to a priming administration of ethanol (0.4 g/kg, i.p.) were quickly tested for reinstatement of CPP. The time spent by the mice in each chamber was recorded during 15 min (Fig. 1).

*Effects of drug treatment on locomotor activity*

Locomotion was gauged in a CPP apparatus with two chief chambers. The floor of each compartment was separated into 6 equal-sized squares. Locomotor activity was measured for 15 min for each CPP test simultaneously. Locomotion was gauged as the number of crossings from one space to another.

*Effects of drug treatment on motor coordination*

The rotarod test was performed 15 min after post-conditioning tests. After the locomotion test, the animals were

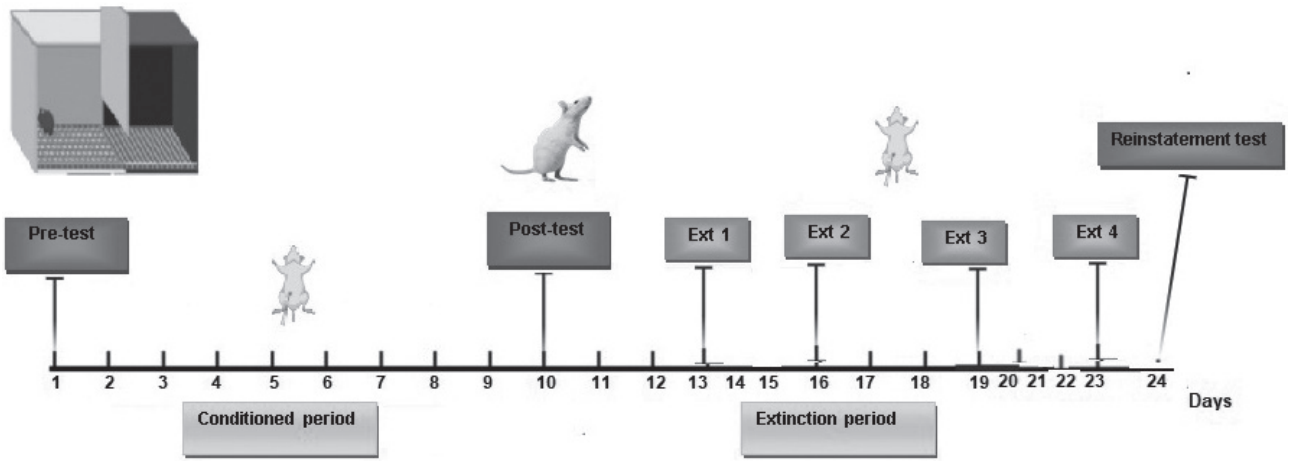


**Figure 1**. Experimental procedures for the whole conditioned place preference process. EXT, extinction phase.

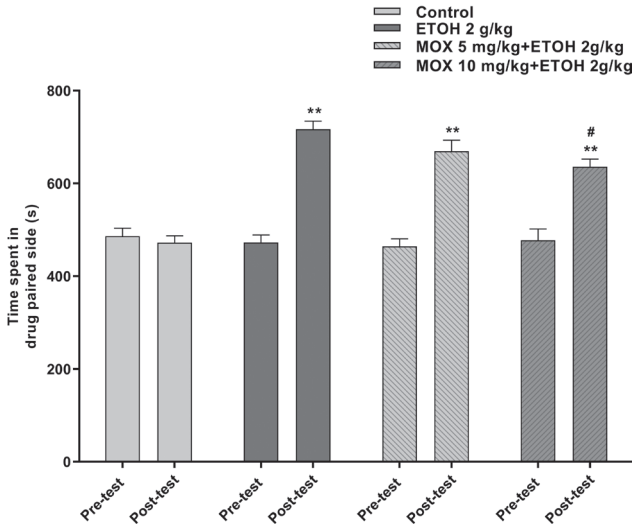


**Figure 2**. Moxidectin effects on the acquisition of ethanol-linked CPP. Co-administration of moxidectin (5 and 10 mg/kg, i.p.) can inhibit the acquisition of alcohol-induced CPP indicated by two way-ANOVA analysis and Bonferroni's test. Data are represented as means ± SEM. ** $p < 0.001$, compared to the control group; $^{\#}$ $p < 0.05$, compared to ETOH group. CPP, conditioned place preference; ETOH, ethanol; MOX, moxidectin; Pre-test, pre-conditioning test; Post-test, post-conditioning test.

slowly put on the rotor with the body axis perpendicular to the rotor head directed contrary to the direction of the rotating rod (5 rpm) and the time to fall from the rod was recorded for each mouse. Four trials were performed, the first two tests were "training," and the other two trials were sequentially carried out for analysis, with a maximum time of 5 min.

*Statistical analysis*

Statistical analyses of the obtained data were performed with the statistical software package Graphpad Prism. Data were analyzed with one- and two-way ANOVA and Bonferroni *post-hoc* test. Data are expressed as mean ± SEM. A difference of $p < 0.05$ between experimental groups was deemed statistically significant.

## Results

*Moxidectin effects on the acquisition of alcohol-induced CPP*

Application of one-way ANOVA showed that ethanol (2 g/kg) produces CPP for the drug-paired chamber [$F(3, 28) = 13.49$, $p < 0.001$] (Fig. 2). This confirms that the CPP was effectively created. Two way-ANOVA analysis indicated significant effects of time [$F(3, 56) = 23.07$, $p < 0.001$], treatment [$F(1, 56) = 300.7$, $p < 0.001$], and time × treatment [$F(3, 56) = 38.64$, $p < 0.001$]. The later analyses (*post hoc* Bonferroni) indicated that moxidectin (10 mg/kg, $p < 0.01$) significantly decreased the time spent in the alcohol-paired compartment compared with the group treated with alcohol. Nevertheless, no statistical differences were seen in the treatment using one dose of moxidectin (5 mg/kg, $p > 0.05$). These results show that pretreatment with moxidectin (10 mg/kg, i.p.) reduced the acquisition of alcohol-linked CPP (Fig. 2).

*Moxidectin effects on extinction of ethanol-induced CPP*

As shown in Figure 3, one-way ANOVA revealed CPP was sufficiently created in animals from all ethanol groups [$F(3, 28) = 64.57$, $p < 0.001$]. Two way-ANOVA analysis indicated

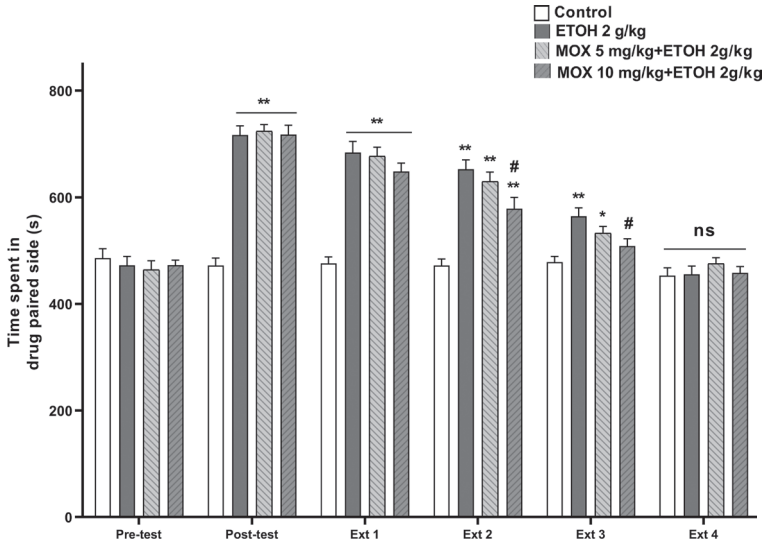


**Figure 3.** Effects of different doses of moxidectin on the extinction (EXT) of ethanol-linked CPP. Moxidectin (5 and 10 mg/kg, i.p.) significantly accelerated the extinction of ethanol-induced CPP. Statistical analysis was performed with repeated-measures ANOVA and Bonferroni's test. Data are represented as means ± SEM. ANOVA followed by Bonferroni's test was applied to ascertain whether there were any significant variations between the groups on every day in the extinction phase. * $p < 0.05$, ** $p < 0.001$, compared to the control-paired group; $^{\#}$ $p < 0.05$, compared to ETOH group. n.s. non-significant. For more abbreviations, see Figure 2.

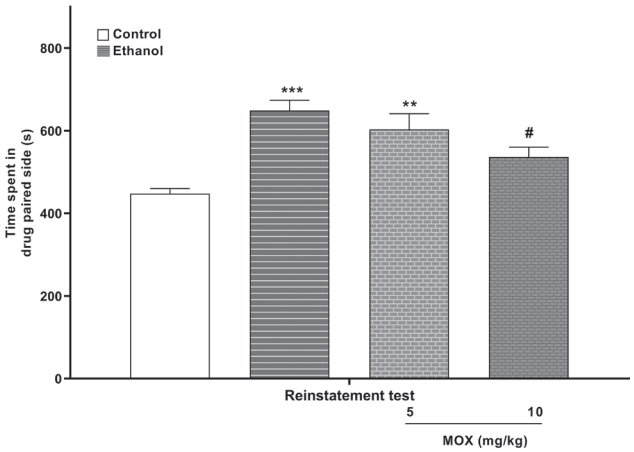


**Figure 4.** Moxidectin effects on drug-priming reinstatement of ethanol-linked CPP. After the extinction test, the mice were administered moxidectin (5 and 10 mg/kg, i.p.) as indicated, and 30 minutes later received the priming dose of ethanol (0.4 g/kg, i.p.). Values are means ± SEM. During the reinstatement tests, data are expressed as time spent in the conditioning compartments calculated for mice in the drug-paired chamber: ** $p < 0.01$, *** $p < 0.001$ compared to the control group; # $p < 0.05$, compared to the ethanol group. For abbreviations, see Figure 2.

significant effects of time [F (12.26) = 54.94, $p < 0.001$], treatment [F (2.480) = 90.11, $p < 0.001$], and time × treatment [F (33,57) = 18.44, $p < 0.001$]. The *post hoc* Bonferroni analysis found that moxidectin significantly extenuated the time spent in the drug-paired compartment with a dose of moxidectin (10 mg/kg, i.p., $p < 0.05$) on extinction day 3 (Fig. 3), and for the same dose of moxidectin (10 mg/kg, i.p., $p < 0.05$) on extinction day 6 when compared to the ethanol group (Fig. 3). On the 9th day of extinction, only the ethanol ($p < 0.001$) and moxidectin ($p < 0.05$) groups were found to be significant compared with the control group. The results show that the extinction of ethanol-linked CPP was created on Ext 12 [i.e. no significant difference was found between the time spent by the ethanol-paired group and the saline-paired group (Fig. 3)].

*Moxidectin effects on reinstatement of ethanol-induced CPP*

To assess if the extinction of CPP was created in mice, two way-ANOVA analysis was performed for the pre-CPP, extinction and saline-treated groups. According to the results, there were insignificant differences in the time spent in the drug-paired chamber in the extinction session and the time spent in the same chamber throughout pre-conditioning (test phase: F [1, 56] = 1.652, $p > 0.05$; group: F [3, 56] = 0.8317, $p > 0.05$; group × test phase interaction: F [3, 56] = 0.08044, $p > 0.05$; Fig. 4). The two-way ANOVA test showed that the extinguished CPP was comprehensively reinstated after injections with a single low dose of ethanol (0.4 mg/kg, i.p.) compared with the saline-primed groups [F (1, 56) = 40.22, $p < 0.001$)] (Fig. 4). *post hoc* Bonferroni multiple comparison test showed that moxidectin (10 mg/kg, $p < 0.01$) significantly decreased the effect of ethanol on CPP compared to the ethanol group. Additionally, a lower dose of moxidectin (5 mg/kg, $p > 0.05$) had no significant effect (Fig. 4).

*Moxidectin effects on locomotor activity in ethanol-induced CPP*

One-way ANOVA showed the effect on locomotor activity was not significant with ethanol [F (3, 22) = 0.6028, $p > 0.05$)]

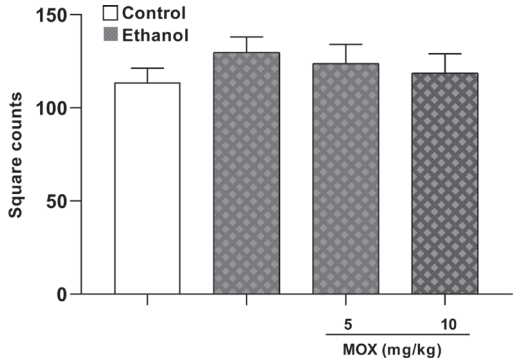


**Figure 5.** Ethanol alone and co-administration with moxidectin (5 and 10 mg/kg, i.p.) did not display any effect on motor activity of mice. Locomotor activity was measured for 15 min after the CPP test. Data are represented as means ± SEM. The results showed no significant difference among raters for all groups ($p > 0.05$). For abbreviations, see Figure 2.

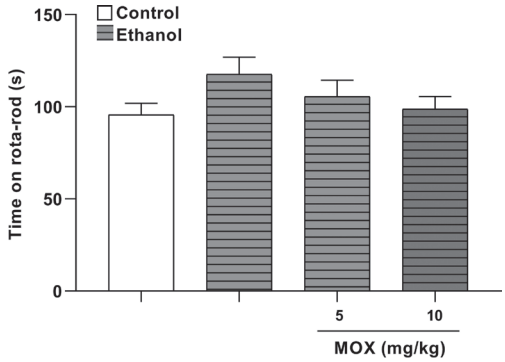


**Figure 6.** Ethanol alone and co-administration with moxidectin (5 and 10 mg/kg, i.p.) did not display any effect on the rotarod test for mice. The test was performed 15 min after the post-conditioning test. Data are represented as means ± SEM. The results showed no significant difference among raters for all groups ($p > 0.05$). For abbreviations, see Figure 2.

(Fig. 5). In addition, locomotor activity was found not to be statistically significant in the moxidectin groups which were also administered ethanol ($p > 0.05$).

*Moxidectin effects on motor coordination of mice*

One-way ANOVA showed the effect on motor coordination was not significant with ethanol [$F(3, 22) = 0.2797, p > 0.05$)] (Fig. 6). In addition, motor coordination was found not to be statistically significant in the moxidectin groups which were also administered ethanol ($p > 0.05$).

## Discussion

The global health and social costs caused by alcohol dependence are substantial and increasing. Alcohol use disorder can result in alcohol-related cardiovascular disease, which is associated with the development of various chronic disorders such as diabetes mellitus, cerebellar degeneration, dementia, fetal alcohol syndrome, hypertension, and other substance use and mental health diseases (Horakova et al. 2016; Rittenberg et al. 2020; Yunusoğlu 2022a). This study demonstrates that exposure to alcohol at a dose of a 2 g/kg induced CPP in mice; findings which are compatible with the literature (Napier et al. 2013; McKendrick and Graziane 2020). Moxidectin at a dose of 10 mg/kg inhibited the acquisition of CPP. Moreover, moxidectin at the same dose was able to rapidly extinguish CPP and prevented the reinstatement of alcohol-induced CPP following the administration of a low priming dose of ethanol (0.4 g/kg). In addition, ethanol alone and co-administration with moxidectin did not change locomotor activity and motor coordination**.** In the present investigation, effective doses for treatment with moxidectin were selected from those determined in previous research studies based on alcohol addiction (Huynh et al. 2017). In previous studies, moxidectin significantly decreased ethanol consumption in rodents in a dose-dependent manner (Huynh et al. 2017). In addition, we showed the reducing effect of moxidectin on nicotine-induced CPP in our previous study (Yunusoğlu et al. 2021). In this study, we provide the first data showing that moxidectin reduces the extinction and relapse of alcohol addiction. Withdrawal and relapse, just like the development of drug and substance dependence, are important details in drug addiction and lead to major problems during the treatment of drug and substance abuse (Napier et al. 2013; Allahverdiyev et al. 2015; McKendrick and Graziane 2020; Yunusoğlu 2021b). These findings demonstrate that moxidectin inhibited alcohol-induced CPP in mice.

Drug-related memories share the same processes as nondrug-associated memories; that is consolidation, reconsolidation, retrieval, conditioning/development, extinction, and reinforcement (Napier et al. 2013; Shen et al. 2020). Extinction is an operative process that commences with a progressive refusal of acquired answers and involves formation of novel memories, which provisionally suppress the expression of the original drug-related memories (McKendrick and Graziane 2020; Shen et al. 2020). In the continuing search for new therapeutics that promote the extinction of drug-related memories, new studies provided favorable results in several laboratory animal models of addiction and dependence (Shen et al. 2020). For the extinction treatment, addicted individuals are exposed to drug-related cues again and again during the withdrawal of drugs and substances, in order to decrease craving, rewarding effects of drug cues, and inhibit relapse. Furthermore, an expanding number of studies indicated that many pre-extinction or post-extinction pharmacological interventions can precipitate extinction of drug-related behavior; therefore, they can decrease the tendency to relapse (Napier et al. 2013; McKendrick and Graziane 2020; Shen et al. 2020).

GABA is the main inhibitory neurotransmitter in the brain and is essentially synthesized from glutamate (Tanchuck et al. 2011; Banerjee 2014; Kranzler et al. 2019). Ethanol enhances this activity by creating an increase in GABA release through GABA-releasing neurons. A series of recent studies indicated that GABA agonists reduce alcohol dependence (Bechtholt and Cunningham 2005; Banerjee 2014; Martinez et al. 2018). It was demonstrated in previous investigations that moxidectin activates GABA receptors (Ménez et al. 2012; Huynh et al. 2017; Spampanato et al. 2018). Moxidectin can contribute to the decrease in ethanol-induced CPP through this mechanism. These results are in line with previous data, as mentioned before (Bechtholt and Cunningham 2005; Banerjee 2014; Martinez et al. 2018).

Glutamate is a primary excitatory neurotransmitter that is released by nerve cells in the brain and stimulated by N-methyl-D-aspartate (NMDA) receptors (Bisaga and Popik 2000; Zmarowski et al. 2005). Previous studies reported that NMDA receptor antagonists have a reducing effect on alcohol addiction and dependence (Biała and Kotlińska 1999; Bisaga and Popik 2000; Krystal et al. 2003; McGeehan and Olive 2003; Nguyen et al. 2011; Kurokawa et al. 2013; Morisot and Ron 2017). It was also reported that moxidectin inhibited glutamate activation expression (Njue et al. 2004; Janko and Geyer 2013). In another study, the anti-craving medication acamprosate was also utilized clinically to maintain withdrawal in detoxified alcoholics (Littleton et al. 2001). The main mechanism primary to the relapse-preventing influence of acamprosate is possibly a reduction in glutamatergic hyperexcitability due to functional antagonism of the NMDA receptor (Littleton et al. 2001). Hence, moxidectin may reduce alcohol-linked CPP *via* the NMDA receptor. These results are similar to those published in several studies, which found ethanol-induced CPP with

NMDA receptor antagonists on average (Biała and Kotlińska 1999; Aguilar et al. 2009; Khan and Pandy 2016).

Inside the mouse central nervous system, there are two main purinergic receptor classes binding adenine nucleotides including adenosine 5-triphosphate, consisting of the P2X class of ligand-gated ion channels and the P2Y class of G protein-coupled receptors (Franklin et al. 2014; Huynh et al. 2017; Huynh et al. 2019). Therefore, purinergic P2X receptors (P2XRs) form functional heteromeric or homomeric receptors (Huynh et al. 2019). P2XRs change the release of different neurotransmitters which may be co-released with ATP. P2X4R was reported to be changed by dopamine depletion (Franklin et al. 2014). Dopamine is the neurotransmitter that was classically linked with the reinforcing effects of a substance(s) such as alcohol and other drugs of abuse and may have an important role in triggering the neurobiological changes associated with addiction (Franklin et al. 2014). Recent studies reported that P2X4Rs can play an influential role in the reward circuitry by regulating the release of dopamine or glutamate within the ventral tegmental area and nucleus accumbens (Franklin et al. 2014; Huynh et al. 2019). These two anatomical areas, dopamine and glutamate are observed to be crucial in alcohol addiction (Franklin et al. 2014). For the first time, Huynh et al. (2017, 2019) demonstrated that moxidectin acts on P2X4Rs. Interpreting these data, moxidectin may decrease ethanol-linked CPP *via* P2X4Rs.

Modern pharmacological strategies for substance and drug dependence or abuse target modulation or inhibition of the effects of a drug at sites of action in the body by reducing three critical points of withdrawal syndrome, craving, and relapse (Allahverdiyev et al. 2015; McKendrick and Graziane 2020). Among pharmacological remedies that are generally used to reduce abstinence symptoms, few can reduce drug or substance craving, and they are seldom effective in preventing relapse as well (Allahverdiyev et al. 2011; McKendrick and Graziane 2020). Various phases of the CPP test mimic real clinical states like acquisition for craving, extinction for withdrawal, and reinstatement for relapse. In this work, moxidectin decreased the development of alcohol craving (acquisition), withdrawal (extinction) syndrome, and relapse (reinstatement).

There are some limitations to this work that should be taken into account. First, the antiparasitic pharmacologic agent moxidectin normally displays limited brain penetration in vertebrates due to efficient drug efflux at the blood-brain barrier by P-glycoprotein, encoded by the multi-drug resistance gene (Janko and Geyer 2013). Since moxidectin, which we used at a low dose in a short time, did not reach sufficient concentrations in the brain, it may have been statistically insignificant. In similar future studies, it may be appropriate to administer moxidectin approximately 60 min or longer before alcohol administration and therefore this effect may be explained by obtaining more study results. Secondly, this work was performed with male mice. Sex variations are seen through all stages of alcohol addiction and dependence, from commencement to dependence, withdrawal, and relapse (Becker and Koob 2016; Cunningham and Shields 2018; Finn 2020). Despite some variations between the subjects which result from methodological problems, females seem to be more sensitive to the conditioned rewarding influences of alcohol than males, in general (Cunningham and Shields 2018). Sex variations in drug and substance usage and abuse, including alcohol addiction, were clearly documented in numerous investigations showing that dependence in females occurs at lower doses than males but takes place more quickly (Becker and Koob 2016; Díaz-Mesa et al. 2016; Cunningham and Shields 2018). This fact is an issue for future investigations to explore. Moxidectin could reduce alcohol-induced CPP *via* various mechanisms. The probable mechanisms may be explained by using multiple receptor blockers or activators in later research. Thirdly, mice are crepuscular animals, that means that they are most active during dawn and dusk. This research was carried out during the light phase. Future CPP investigations of moxidectin may be conducted during dawn and dusk time periods.

The results of this research provide data about the potential therapeutic usage of moxidectin to develop innovative pharmacological strategies for the treatment of alcohol addiction. Moxidectin might be beneficial for the prevention and treatment of alcohol addiction; however, further investigation is required to completely explain this characteristic.

**Conflict of interest.** None of the authors have conflicts of interest concerning this manuscript.

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