

## Resveratrol inhibits nicotine-induced conditioned place preference in mice

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Nicotine addiction leads to a huge burden on public health and the economy worldwide. Resveratrol (3,5,4'-tetrahydroxystilbene) is the most well-known polyphenolic stilbenoid. Resveratrol was shown to exhibit positive effects on numerous mechanisms that are important for drug and substance addiction. Thus, this study aimed to examine the effect of resveratrol on nicotine addiction. Intraperitoneal (i.p.) treatment with nicotine (0.5 mg/kg) significantly enhanced time spent in the nicotine-paired compartment. Resveratrol (50 and 75 mg/kg, i.p.) and varenicline (2 mg/kg, i.p.) co-administered with nicotine during the 3-day conditioning period effectively diminished the acquisition of nicotine-induced conditioned place preference (CPP). On the other hand, the administration of resveratrol (50 and 75 mg/kg, i.p.) and varenicline (2 mg/kg, i.p.) decreased the low dose (0.1 mg/kg, i.p.) nicotine-induced reinstatement. The results suggest that resveratrol and varenicline inhibit the acquisition and reinstatement of nicotine's reward properties. Resveratrol displayed similar results in the CPP phases as obtained with the reference drug varenicline. In conclusion, resveratrol could be beneficial as an adjuvant pharmacotherapy for nicotine addiction; however, more investigation is needed to completely explain this property.

**Keywords:** Nicotine. Resveratrol. Acquisition. Reinstatement. Conditioned place preference.

### INTRODUCTION

Drug and substance addiction is recognized as a disease of the brain reward system, which is considered a multifactorial disorder of the central nervous system (Tiwari *et al.*, 2020). The morbidity and mortality rates as a result of tobacco consumption create a significant burden on health care resources all around the world (D'Souza, 2016). Currently, there are about 1.3 billion adult smokers globally, which makes tobacco dependence one of the most widespread addictions in the world (D'Souza, 2016). Nicotine, the main component of tobacco, produces craving and abstinence symptoms both in animals and humans (Tiwari *et al.*, 2020). Nicotine mainly acts through specific nicotinic acetylcholine receptors located in the brain (Tiwari *et al.*, 2020).

Resveratrol is a stilbenoid generated by many plants in response to damage or to pathogens such as fungi and bacteria (Singh *et al.*, 2019). It exists in various edible parts of plants such as grapes, berries, pistachios, peanuts, and plums. Additionally, it can also be artificially synthesized. Red wine is the most concentrated food source for resveratrol. Resveratrol is a polyphenolic nutraceutical that induces pleiotropic activities in human subjects (Ruivo *et al.*, 2015; Singh *et al.*, 2019). Pharmacological effects described for resveratrol include antiepileptic, anti-inflammatory, antioxidant, anti-tumorigenic, neuroprotective, and hypertensive activities (Repossi, Das, Eynard, 2020; Tian, Liu, 2020). A series of recent studies show that resveratrol displays multiple effects on the central nervous system, such as antidepressant, anticonvulsant, anti-neurodegenerative, antinociceptive, anxiolytic, and sedative effects (Dhir, 2020; Repossi, Das, Eynard, 2020; Singh *et al.*, 2019). Treatment with resveratrol was well tolerated, without severe adverse events, and with no treatment-related effects (Repossi, Das, Eynard, 2020).

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Previous studies showed that resveratrol has effects on N-methyl-d-aspartate (NMDA), gamma-aminobutyric acid (GABA), nitric oxide (NO) systems, and calcium ion channels, which are also linked to nicotine addiction and dependence (Abd El-Fattah *et al.*, 2018; Aydin *et al.*, 2017; Barone *et al.*, 2019; Calleri *et al.*, 2014; Chong *et al.*, 2015; Hsieh *et al.*, 2019; Miller *et al.*, 2013; Park *et al.*, 2012; Repossi, Das, Eynard, 2020; Rezaee *et al.*, 2020; Shen *et al.*, 2020; Wang *et al.*, 2016; Wang *et al.*, 2019). A literature survey indicates that NMDA antagonists demonstrate a decreasing effect on the acquisition of nicotine-induced CPP (Li *et al.*, 2014; Yararbas *et al.*, 2010). It was also indicated in a previous study that GABA receptor agonists, PPAR agonists and calcium channel blockers attenuate the reinstatement of nicotine-induced CPP (Aguilar, Rodríguez-Arias, Miñarro, 2009; Biala, Budzynska, 2008; Blanco-Gandía *et al.*, 2018; Fattore *et al.*, 2009; Jackson *et al.*, 2017; Kutlu *et al.*, 2018; Le Foll *et al.*, 2013; Matheson, Le Foll, 2020; Panlilio, Justinova, Goldberg, 2013). Moreover, previous research illustrated that resveratrol diminishes morphine-induced CPP, morphine dependence, and methamphetamine-induced hyperactivity and dopamine overflow in rodents (Han *et al.*, 2014; Miller *et al.*, 2013; Rezaee *et al.*, 2020). Resveratrol has multiple mechanisms that play key roles in nicotine addiction and dependence.

Varenicline, which is a smoking cessation medication approved by the Food and Drug Administration, acts as a partial agonist for  $\alpha 4\beta 2$  nicotinic acetylcholine receptors (nAChR), with a lower order of magnitude of effect on other nAChR subtypes (Gubner, McKinnon, Phillips, 2014). In rodents, varenicline prevents nicotine-induced CPP, decreases nicotine-induced locomotor sensitization, prevents nicotine's effects on brain stimulation reward in intracranial self-stimulation, self-administration under fixed circumstances and suppresses nicotine-priming (Biala, Staniak, Budzynska, 2010; Jordan, Xi, 2018). Nonetheless, violent side effects were reported in patients receiving varenicline concerning adverse cardiovascular effects and neuropsychiatric effects such as depression, suicidal ideation, suicide attempts, and completed suicide (Napier, Herrold, de Wit, 2013). Hence, there is a requirement to develop novel pharmacological

agents with low side effects for the treatment of nicotine addiction and dependence.

The CPP model is widely applied in preclinical and clinical pharmacology, behavioral science, and neuroscience studies (Golden, Jin, Shaham, 2019; Tzschentke, 2007). The CPP paradigm was not only used as a screening tool for drug abuse potential, but was applied to investigate neurotransmitters, brain areas, genes, signaling pathways, and different mechanisms mediating the rewarding (or aversive) effects of drugs (Golden, Jin, Shaham, 2019; Tzschentke, 2007). In CPP studies, biased versus unbiased research designs can be used (Golden, Jin, Shaham, 2019; Tzschentke, 2007).

As can be clearly seen from the abovementioned facts, it was hypothesized that resveratrol will play a beneficial role in the prevention of nicotine addiction.

## MATERIAL AND METHODS

Adult male Swiss albino mice weighing 25-30 g upon arrival were housed in pairs in a colony room. The mice were kept in a 12 h light/dark cycle with water and food presented ad libitum. All experiments were conducted pursuant to the animal protocols as sanctioned by the Institutional Animal Care and Use Committee of Van Yüzüncü Yıl University. Four mice were housed per Plexiglass cage. The mice were randomly assigned to different groups for the experiments (6-8 mice per group). The mice were used only once. Measures that reduced distress to the animals were put in place. All the tests were carried out during the light phase.

### Drugs

Nicotine hydrogen tartrate and varenicline tartrate were dissolved in sterile physiological saline (0.9% NaCl) quickly prior to use. The pH of nicotine solutions was adjusted to 7.4 with NaOH. Doses are expressed as the free base of the drug. All substances were purchased from Sigma Chemicals (St. Louis, MO, USA) and were given intraperitoneally (i.p.) in a volume of 10 mL/kg. Normal saline (0.9% NaCl) was used as control. All drugs were administered at room temperature.

## Nicotine conditioned place preference

### Apparatus

The CPP apparatus comprised 2 Plexiglas square chambers with a similar size (20 cm long×20 cm high×20 cm wide). To provide a tactile difference between the chambers, one of the compartments had a mesh sheet floor, while the other one had a grid rod floor and white walls. Chambers were cleaned with ethanol and dried between tests to prevent interference by odors created by urine and/or feces.

### Handling habituation

One day prior to handling, mice were singly housed. Handling and habituation were carried out on the 3 days before the start of testing. Initially, mice were put into a dimly lit room at 09:00 AM and allowed to habituate for four hours. Mice were later placed back in their home cages and left to habituate to the room until 4:00 PM, at which point they were allowed to return to their rooms.

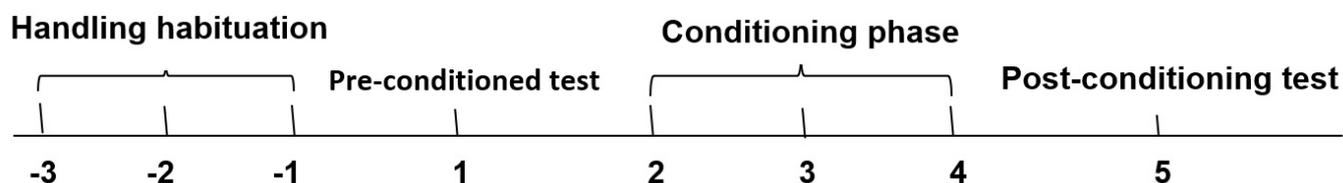
### Pre-conditioning test

On day 1, mice were taken to the scheme room and placed alone on the central line for 5 min. Subsequently,

after the 5-min habituation time, the sliding doors were opened, and they were allowed to voluntarily travel through the CPP apparatus for 15 minutes while being video recorded. Mice expressing a strong unconditioned choice (more than > 66% of the session) or aversion (less than < 33% of the session) for both chambers were excluded from the research (Biala, Staniak, Budzynska, 2010; Titomanlio, Perfumi, Mattioli, 2014).

### Conditioning phase

An unbiased CPP paradigm was applied in this study. During days in the conditioning period, the saline group injected with saline were placed in both chambers and drug groups injected with nicotine were placed in one chamber and saline in the opposite chamber. The CPP protocol used here conforms with prior studies with minor modifications. Mice were put in the related chamber by separating it with a sliding door. Mice received saline and were enclosed in the saline-paired chamber for 30 minutes (on days 2-4). Four hours later, the same mice received nicotine and were confined to the nicotine-paired side. Nicotine was received in the afternoon session to avoid confounding effects of acute nicotine abstinence on the saline conditioning session (Biala, Staniak, Budzynska, 2010; Titomanlio, Perfumi, Mattioli, 2014). The scheme is demonstrated in Figure 1.



**FIGURE 1** - Conditioning scheme and time program for the nicotine-induced conditioned place preference study.

### Effects of resveratrol on acquisition (development) of nicotine-induced CPP

To study the influence of varenicline and resveratrol on the acquisition (development) of nicotine-induced CPP, the mice were treated with varenicline (2 mg/kg, i.p.) and resveratrol (25, 50, and 75 mg/kg, i.p.), or its vehicle 30 minutes before each nicotine treatment

injection throughout the conditioning test, as defined above (Figure 1).

### Post-conditioning test

Mice were not injected on the test day (on day 5). Animals were allowed access to the entire CPP apparatus for 15 minutes, and the time spent in any chamber during

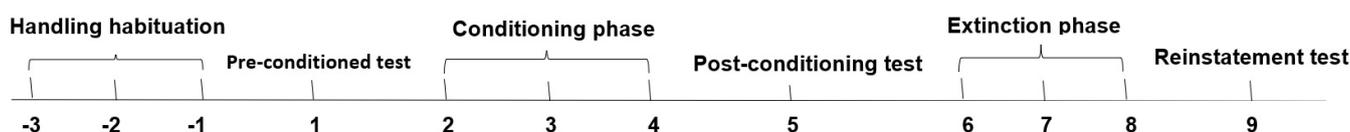
this 15-min duration was noted; data are presented as the time spent on the drug-paired side compared with time spent on the nicotine and saline-paired sides (Biala, Staniak, Budzynska, 2010; Yusoff *et al.*, 2018).

### Extinction of nicotine-induced CPP

Animals were conditioned with nicotine for 3 days and tested for preference on the subsequent day as explained above. Mice were then measured for preference every twenty-four hours without any injection until insignificant preference was observed.

### Effects of resveratrol on drug triggering reinstatement of nicotine-induced CPP

To investigate the influences of varenicline and resveratrol on the reinstatement of CPP, CPP was induced in mice (as described above). One day after the last extinction trial, mice were injected with resveratrol (25, 50, and 75 mg/kg, i.p.), varenicline (2 mg/kg, i.p.) or saline, 30 min before a priming injection of nicotine (0.1 mg/kg, i.p.), and were quickly tested for reinstatement of CPP (Figure 2). Throughout this reinstatement test, each mouse was allowed free access to travel through the conditioning chambers for 15 min (Jackson *et al.*, 2017; Titomanlio, Perfumi, Mattioli, 2014).



**FIGURE 2** - Drug-priming reinstatement scheme and time program for the nicotine-induced conditioned place preference study.

### Measurement of effects of resveratrol treatment on motor coordination

The purpose of the test is to assess the sensorimotor coordination and grip strengths of mice. The rotor was separated into two chambers which allowed two mice to be tested concurrently. For the motor coordination test, the mice were softly placed on the rotor with the body axis perpendicular to the rotor's long axis including the head directed contrary to the direction of the turning rod (5 rpm) and the time to fall off the rod was recorded for every mouse. Five trials were completed, 2 trials were "training," and the other 3 trials were sequentially completed for analysis, with a maximum time of 300 s (Tzschentke, 2007).

### Measurement of effects of resveratrol treatment on locomotor activity

Locomotor activity was determined based on a procedure used during the post-conditioning test in a drug-free state (Tzschentke, 2007). Locomotor activity

was determined in the two main chambers with the floor of the apparatus divided into six equal-sized squares. Locomotor activity was determined as the number of crossings from one square to another during 15 min.

### Statistical analysis

Results are expressed as mean change in preference (s)  $\pm$  SEM (Prism software, GraphPad). Data were analyzed with one-way analysis of variance (ANOVA) followed by post hoc Bonferroni's multiple comparison test.  $P < 0.05$  was considered statistically significant in all cases.

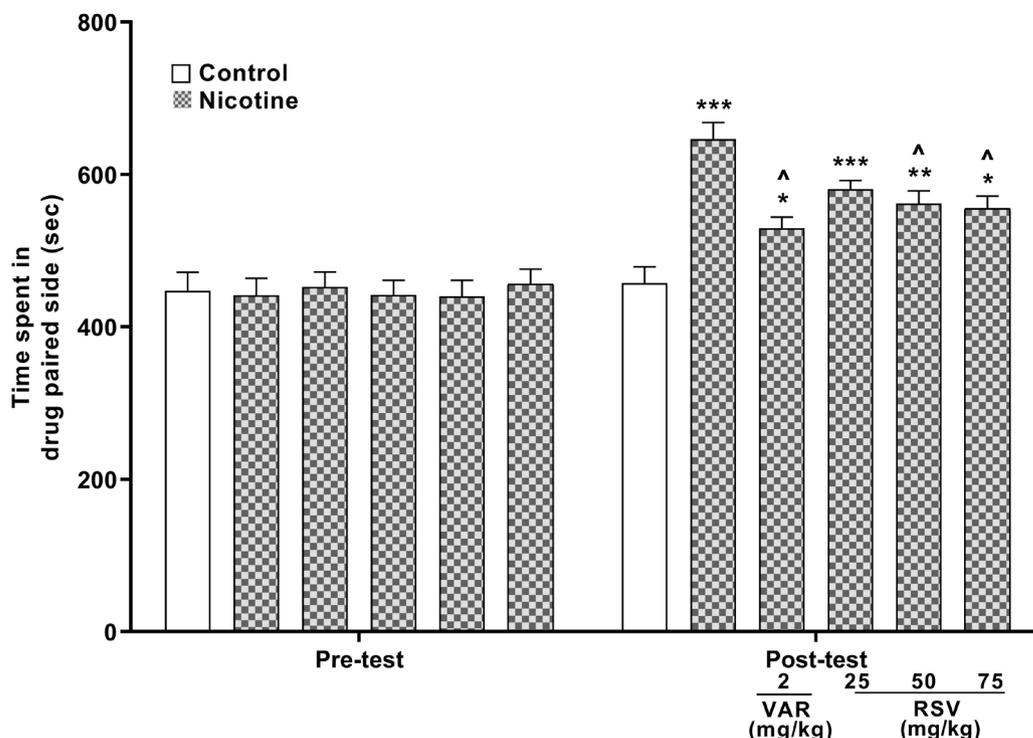
## RESULTS

### Resveratrol inhibited acquisition of nicotine-induced CPP

Treatment with nicotine significantly enhanced the place preference for the drug-paired chamber ( $p < 0.001$ ; see Figure 3). ANOVA showed that varenicline (2 mg/

kg) and resveratrol (50 and 75 mg/kg, i.p.) significantly diminished the place preference for the nicotine (0.5 mg/kg, i.p.) paired compartment [ $F(5, 32) = 12.39$ ;  $p < 0.05$ ]. Saline treatment in the conditioning chamber did not produce either preference or aversion ( $p > 0.05$ ). Post hoc Bonferroni's multiple comparison test showed that

varenicline (2 mg/kg, i.p.) and resveratrol (50 and 75 mg/kg, i.p.) significantly reduced the effect of nicotine on CPP compared to the nicotine treated mice ( $p < 0.05$ ,  $p < 0.05$  and  $p < 0.05$ , respectively). In addition, the lower dose of resveratrol (25 mg/kg, i.p.) had no significant effects ( $p > 0.05$ ).

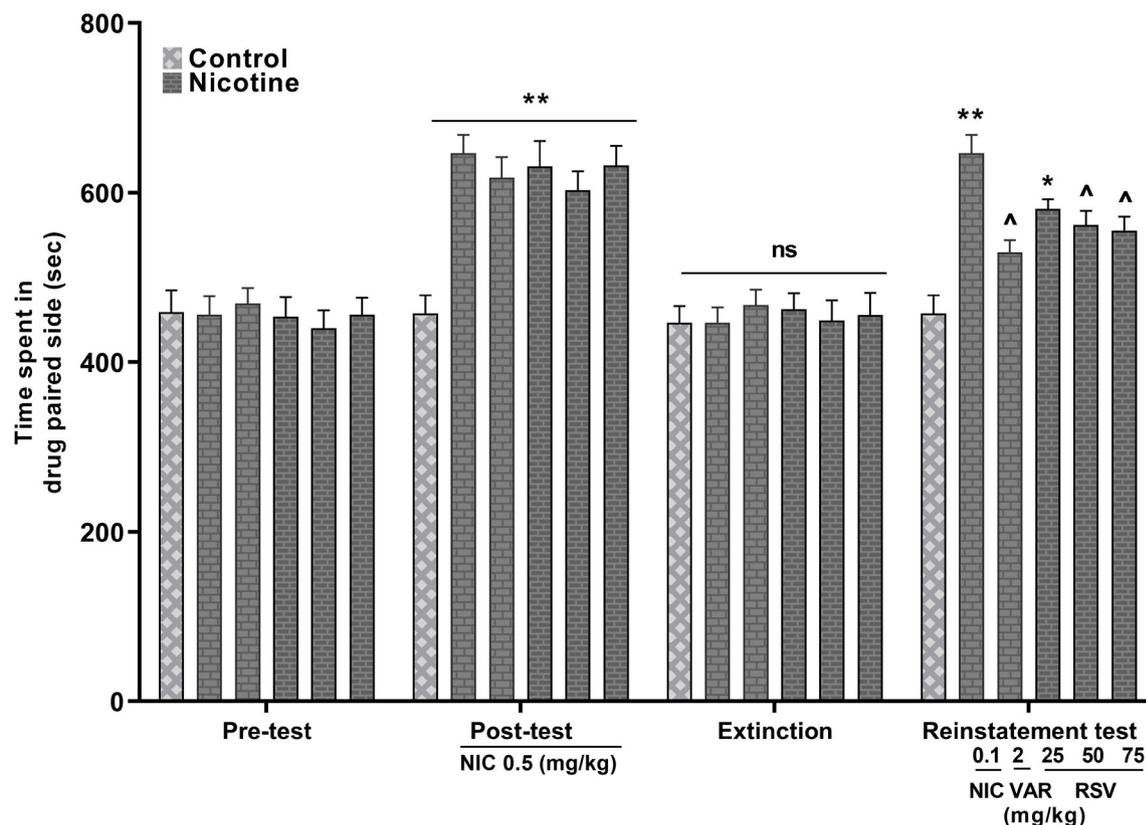


**FIGURE 3-** Effect of resveratrol on nicotine-induced conditioned place preference (CPP). The animals received varenicline (2 mg/kg, i.p.), resveratrol (25, 50, and 75 mg/kg i.p.) or saline, 30 minutes before nicotine (0.5 mg/kg; i.p.) treatment during the acquisition phase. Data given as means ( $\pm$  SEM) of time spent in drug-associated compartments during the 15 min test session. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.01$  compared to the saline group; ^ $p < 0.05$  compared to the nicotine group. Varenicline - VAR, Resveratrol - RES.

### Resveratrol inhibited reinstatement of nicotine-induced CPP

The influence of varenicline and resveratrol on low dose nicotine (0.1 g/kg, i.p.) priming induced CPP is presented in Figure 4. One-way ANOVA showed that nicotine developed place preference for the drug-paired chamber [ $F(5,32) = 9.402$ ,  $p < 0.001$ ]. Bonferroni test indicated that the time spent in the drug-paired side

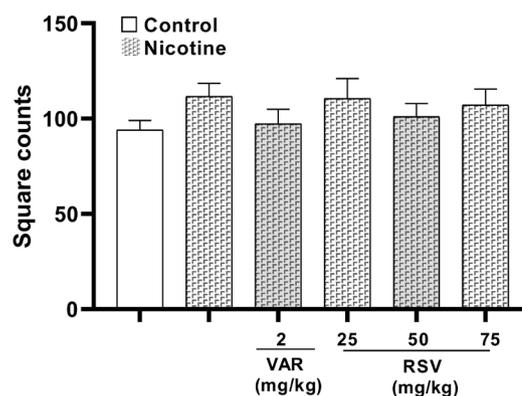
during reinstatement with a priming low dose of nicotine (0.1 mg/kg, i.p.) a day later was significantly ( $p < 0.01$ ) increased when compared to the time spent in the nicotine and saline-paired side. Post hoc Bonferroni's multiple comparison tests demonstrated that varenicline (2 mg/kg, i.p.) and resveratrol (50 and 75 mg/kg, i.p.) significantly reduced the effect of nicotine on CPP compared to the nicotine treated mice ( $p < 0.05$ ,  $p < 0.05$ , and  $p < 0.05$ , respectively).



**FIGURE 4** - Nicotine-induced reinstatement of conditioned place preference. After the extinction test, the mice were treated with varenicline (2 mg/kg, i.p.), different doses resveratrol (25, 50, and 75 mg/kg, i.p.) and saline, as indicated, 30 minutes before the priming-nicotine administration (0.1 mg/kg, i.p.). Data are given as means ( $\pm$  SEM) of time spent in drug-associated compartments during the 15-minute test session. \*\* $p < 0.001$ , \* $p < 0.01$  compared to the saline group; ^ $p < 0.05$  compared to the nicotine group. Varenicline - VAR, Resveratrol - RES, Nicotine - NIC.

#### Nicotine alone or in combination with resveratrol did not influence the motor activity

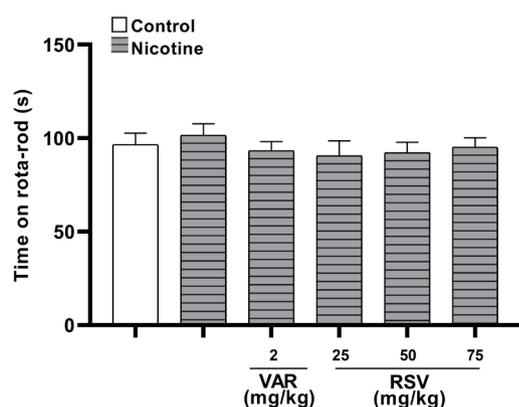
One-way ANOVA showed that nicotine did not cause any effect on locomotion during the test session [ $F(5, 32) = 0.9019$ ;  $p > 0.05$ ]. Post hoc analysis shows the influence of varenicline (2 mg/kg, i.p.) and various doses of resveratrol (25, 50 and 75 mg/kg, i.p.) had no influence on locomotion while treated during the acquisition of nicotine-induced CPP, as in Figure 5.



**FIGURE 5** - Effect of varenicline (2 mg/kg, i.p.) and resveratrol (25, 50, and 75 mg/kg, i.p.) on locomotor activity in the acquisition of nicotine-induced CPP. Locomotion was measured based on a method appropriated before through the post-conditioning test in a drug-free status. Locomotion was measured in chambers with the floor of the CPP apparatus separated into 6 equal-sized squares. Locomotor activity was assessed as the number of crossings from one square to another within 15 minutes. Values are means  $\pm$  SEM. There was no significant difference among groups ( $p > 0.05$ ). Varenicline - VAR, Resveratrol - RES.

### Nicotine alone or in combination with resveratrol did not influence the motor coordination

ANOVA showed that the nicotine did not induce any influence on motor coordination during the test session [ $F(5, 32) = 0.4477$ ;  $p > 0.05$ ]. Post hoc analysis indicates the influence of varenicline (2 mg/kg, i.p.) and various doses of resveratrol (25, 50 and 75 mg/kg, i.p.) had no effect on motor coordination while treated during the acquisition of nicotine-induced CPP, as in Figure 6.



**FIGURE 6** - Effect of varenicline (2 mg/kg, i.p.) and resveratrol (25, 50, and 75 mg/kg, i.p.) on the rotarod test. Five trials were performed, two trials were “training,” and the other three trials were sequentially conducted for the report, with a maximum time of 300 seconds. Values are means  $\pm$  SEM. There was no significant difference among groups ( $p > 0.05$ ). Varenicline - VAR, Resveratrol - RES.

## DISCUSSION

The general current pharmacological strategies against drug and substance addiction/dependence are to attenuate or prevent the effects of the drug at sites of action in the body by reducing three principal aspects: abstinence/withdrawal syndrome, craving, and relapse (You *et al.*, 2019). Among the pharmacological treatments that are usually utilized to minimize abstinence symptoms, few can reduce the drug craving, and they are also rarely effective in preventing relapse (Allahverdiyev, Nurten, Enginar, 2011; Tzschentke, 2007).

The present investigation examined the effects of varenicline and resveratrol on the rewarding properties of nicotine as calculated by following several phases of

CPP. Nicotine-conditioned mice showed CPP clearly as increased amount of time spent in the drug-associated chamber relative to saline controls, which is consistent with the outcomes of previous research. This study demonstrates that varenicline and resveratrol can significantly diminish the acquisition and reinstatement characteristics of nicotine. Resveratrol displayed a similar result in the CPP phases compared to the reference drug varenicline. There was no statistical significance in all determinations for locomotor activity and rotarod. These results are in line with the literature (Fartootzadeh *et al.*, 2019; File, Cheeta, Akanezi, 2001; Sahraei *et al.*, 2004; Titomanlio, Perfumi, Mattioli, 2014). In this research, the administered doses of resveratrol and nicotine were chosen from the effective doses determined based on a previous study about morphine and nicotine-induced CPP (Biala, Staniak, Budzynska, 2010; Rezaee *et al.*, 2020).

Dopaminergic pathways are important structures that modulate reward systems (Tiwari *et al.*, 2020). Various examinations confirmed that NMDA receptor blockers have a decreasing effect on dopamine levels in the brain’s reward area and diminishing effect on nicotine addiction (Blokhina *et al.*, 2005; Kaminski *et al.*, 2011; Wang *et al.*, 2010). The literature survey showed that resveratrol attenuates glutamate activation on NMDA (Hsieh *et al.*, 2019; Wang *et al.*, 2019). It was indicated in numerous studies that NMDA antagonists decrease the acquisition and expression of nicotine-induced CPP (Li *et al.*, 2014; Yazarbas *et al.*, 2010). This suggests that these results are similar to those obtained from other investigations, in which the inducing effect on CPP were generally reported with NMDA receptor antagonists.

GABA is produced in brain cells from glutamate, and acts as a principal inhibitory neurotransmitter. Nicotine changes GABA activity in the brain by various mechanisms (D’Souza, 2016). It was also found that GABA agonists decrease nicotine dependence, and reinstatement, and also accelerate the extinction of nicotine-conditioned place preference (Biala, Budzynska, 2008; Fattore *et al.*, 2009; Kutlu *et al.*, 2018; Li *et al.*, 2012; Paterson, Froestl, Markou, 2004; Varani *et al.*, 2014). Furthermore, a series of recent studies show that resveratrol stimulates GABA. This suggests that the results obtained from this study are similar to those

obtained from other studies, in which the inducing effect on CPP were generally reported with GABA receptor agonists.

Lately, the role of PPARs in drug and substance addiction has gained attention (Domi *et al.*, 2019). A literature review indicates that PPAR agonists attenuate nicotine addiction (Jackson *et al.*, 2017; Matheson, Le Foll, 2020). Besides, a series of recent studies indicated that resveratrol stimulates PPARs (Barone *et al.*, 2019; Jardim *et al.*, 2018; Nakata, Takahashi, Inoue, 2012). Resveratrol can contribute to the diminished effect of nicotine-induced CPP by using the PPARs activation system. This indicates that these results are in accordance with the those reported in other works, in which the inducing effect on CPP with PPARs receptor antagonists were generally reported.

It was reported that inhibition of nitric oxide synthase (NOS) in rodents diminishes nicotine consumption and, subsequent to chronic nicotine treatment, tolerance and withdrawal symptoms (Sahraei *et al.*, 2004). Previous studies emphasized a decrease in NO level with resveratrol treatment (Aydin *et al.*, 2017; Chong *et al.*, 2015; Xia, Förstermann, Li, 2014). This reduction with resveratrol may contribute to reducing nicotine-induced CPP.

It was suggested in previous studies that calcium channels act in several drug reward and addiction processes (Biala, Budzynska, 2006; Ma *et al.*, 2013; Padula *et al.*, 2015). Prior research suggested the influence of calcium channel antagonists on attenuation of drug dependence, addiction and reinstatement of nicotine-conditioned (Biala, Budzynska, 2006; Biala, Budzynska, 2008; Padula *et al.*, 2015). The majority of prior research using resveratrol showed it exhibits antagonist effects on calcium channels (Lu *et al.*, 2019; Nalli *et al.*, 2016; Yin *et al.*, 2017). Resveratrol may diminish nicotine-induced CPP by affecting calcium channels.

Regarding pharmacological medication plans for drug addiction, some modern investigations focused on and highlighted the effectiveness of natural products in neutralizing various kinds of drug addiction and dependence, including nicotine, opioid, cocaine methamphetamine, and alcohol dependence (Lu *et al.*, 2009; Mendes, Prado, 2016). Considerable modern drugs

are obtained from natural product sources, which have a meaningful function in drug development applications in the pharmaceutical industry. There was a reappearance of interest in natural medicines (Thomford *et al.*, 2018; Veeresham, 2012). Consequently, relapse is the primary point of drug addiction, as well as the principal problem in the treatment of drug abuse (or substance abuse) (Koob, Volkow, 2016; Thomford *et al.*, 2018; Zou *et al.*, 2017). Different factors can augment craving and the subsequent vulnerability to relapse following detoxification.

Various experimental and clinical investigations confirmed that re-exposure to the drug (priming) is an extremely critical event associated with drug-seeking behavior both in humans and in animal addicts (Aguilar, Rodríguez-Arias, Miñarro, 2009). As resveratrol has powerful pharmacological influences on addiction mechanisms, it may diminish the nicotine-induced CPP. Besides, the influence of resveratrol on nicotine addiction was not investigated yet. Therefore, since diminished relapse is the main goal of the drug (or substance) dependence medication and it is still the main limitation in drug therapy, we additionally employed the paradigm of CPP to evaluate the role of resveratrol in the reinstatement of drug-seeking (or drug craving) behavior induced by priming with low dose nicotine (Napier, Herrold, de Wit, 2013).

There are also some limitations to this work that must be considered. First, this study used male mice. Sex differences are present for all phases of nicotine dependence (initiation, increase of use, dependence, and relapse following withdrawal) (Pogun *et al.*, 2017; Yazarbas *et al.*, 2010). Female rodents are less likely to atone for an enhanced unit dose by reducing response and consumption rates of nicotine. Despite some differences between the studies resulting from methodological problems, overall males appear to be more sensitive than females to the conditioned rewarding effects of nicotine. Sex differences in nicotine addiction were clearly demonstrated in multiple studies showing that females start at lower doses than males, but become dependent quicker (Pogun *et al.*, 2017; Yazarbas *et al.*, 2010). This remains a question for research and researchers can evaluate their categories of observation in their examination. Secondly, in this study the combination

of varenicline, which was used as a reference drug, with resveratrol was not investigated. Lower doses of resveratrol may be effective when used in combination with varenicline. This combination can be utilized as a research subject for future studies.

In summary, these results are the first data about the potential therapeutic usage of resveratrol to develop novel pharmacological approaches for adjuvant treatment of nicotine addiction. According to the results, it can be concluded that resveratrol may be beneficial in the adjuvant treatment of both nicotine and other types of addiction; however, more investigation is required to completely explain this feature.

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## CONFLICTS OF INTEREST

There is no conflict of interest about the submitted study article.

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