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by Samirah Samirah

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Research Article

Andrographolide and Epigallocatechin Gallate (EGCG) Lower the Risk of Addiction Induced by Nicotine and Cigarette Smoke Extract (CSE) in Mice

Samirah Samirah[1], Kurnia Indrayanti[1], Ahmad Dzulfikri Nurhan[1], Vida Softyana[1], Nadya Ratri Pradipta[1], Chrismawan Ardianto[1], Yulistiani Yulistiani[1], Mahardian Rahmadi[1]*

[1]*Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, Indonesia.*

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Abstract

Background: Nicotine, a psychoactive compound from the tobacco plant, produces a reward effect that potentially causes addiction. It is postulated that nicotine addiction occurs through increased reactive oxygen species production in nucleus accumbens, which causes damage to the endogenous antioxidant defense system resulting in an increased need for nicotine intake, which leads to addiction. The antioxidants, andrographolide and epigallocatechin gallate (EGCG), are expected as potential substances to decrease the risk of nicotine addiction. This study aimed to analyze the effect of andrographolide and EGCG on the risk of addiction induced by nicotine and cigarette smoke extract (CSE) in mice.

Methods: Thirty-five Balb/c male mice, divided into seven groups, were used in this study. The administered drugs were normal saline 10 mL/kg BW as control group, nicotine 0.5 mg/kg BW, CSE 1.0 mg/kg BW, andrographolide 50 mg/kg BW, and EGCG 50 mg/kg BW as pre-treatment. Conditioned place preference (CPP) with a biased design method was used to evaluate the reward effects induced by nicotine and CSE. Several stages were carried out, namely pre-conditioning, conditioning, post-conditioning, extinction, and reinstatement tests.

Results: Based on the CPP score, both nicotine ($p<0.001$) and CSE ($p<0.001$) groups increased the reward effect significantly compared to that of the normal saline group. The andrographolide + nicotine ($p<0.001$) and EGCG + nicotine ($p<0.001$) groups decreased the reward effect significantly compared to that of the nicotine group without pharmacological treatment. Similarly, the andrographolide + CSE ($p<0.001$) and EGCG + CSE ($p<0.01$) groups decreased the reward effect significantly compared to that of the CSE group without pharmacological treatment.

Conclusion: Andrographolide and EGCG lower the risk of addiction induced by nicotine and CSE.

Introduction

Tobacco smoking remains a complex global health problem worldwide.[1,2] A study released by the Center for Disease Control and Prevention (CDC) reveals that the exposure to tobacco smoking, especially conventional cigarettes, causes about 480,000 deaths each year, 40% due to cancer, 35% due to heart disease and stroke, and 25% due to lung cancer.[1] Another study revealed that smoking-related diseases were responsible for more than 40 million deaths in Canada, the United Kingdom, and the United States cumulatively from 1960 to 2020.[2] These studies showed that high consumption of tobacco smoke results in a higher burden of disease and death from smoking.

Several studies have confirmed that tobacco smoke contains no less than 7000 compounds. About 250 compounds are classified as carcinogenic or harmful compounds.[1,3] Nicotine is an alkaloid that has a psychoactive activity, which is commonly found in tobacco plants.[4] Continuous nicotine exposure physiologically causes addiction and compulsive behavior to obtain nicotine.[5] Nicotine addiction occurs because nicotine exposure causes activation of nicotinic acetylcholine receptors (nAChRs) on dopaminergic neurons to increase dopamine release in the mesocorticolimbic system.[6]

The stages of nicotine addiction are divided into three stages, namely the acquisition, withdrawal, and reinstatement stages. The acquisition stage is when nicotine exposure induces a reward effect because the stimulation of central nAChRs by nicotine causes the release of dopamine in the mesocorticolimbic area, corpus striatum, such as feelings of pleasure, euphoria, and feelings of calm which play a crucial role in the initiation of smoking habits.[7-9] In

*Corresponding Author: Mahardian Rahmadi, E-mail: mahardianr@ff.unair.ac.id

the withdrawal stage, when desensitization of nAChRs has occurred, then nicotine exposure is reduced or stopped, causing adverse effects such as anxiety, depression, difficulty in concentrating, insomnia, or dysphoria due to decreased responsiveness associated with stimulation of the reward effect by nicotine.[6,9] Then, the reinstatement stage is when nicotine exposure is inhibited after some time. The stimulus in the form of nicotine exposure again results in the desire to get nicotine again, representing a relapse.[6,9]

In a cocaine addiction study, increased production of reactive oxygen species (ROS) caused a decrease in endogenous antioxidant activity indirectly through an increase in dopamine in the mesocorticolimbic system. It led to oxidative stress (OS).[10] The same thing happened to nicotine, where nicotine exposure resulted in ROS production that contributed to drug addiction. *In vivo* studies have shown that nicotine with low concentrations (0.1 μM) induces a significant increase of ROS in mesencephalic cells.[11] The administration of nicotine to mice decreased the activity of superoxide dismutase (SOD), an antioxidant enzyme, significantly, thereby increasing the production of ROS and causing OS.[12] Increased ROS, if not balanced with endogenous antioxidants, such as SOD, may result in desensitization of nAChRs and is hypothesized to have implications for increased risk of nicotine addiction.[10,13]

Andrographolide is an active compound derived from the plant *Andrographis paniculata*, which has many uses, one of which is a natural antioxidant. These compounds are distributed across the blood-brain barrier (BBB) and act as neuroprotective. In one study, the administration of andrographolide increased SOD activity. It decreased ROS production in the mitochondria of the brains of rats exposed to nicotine.[14] Epigallocatechin gallate (EGCG) is the most potent antioxidant of catechins and is also known as a free-radical scavenger. The ability of EGCG as a free-radical scavenger is proven by its ability to extinguish OH radicals 100 times more effectively than mannitol, a typical OH radical scavenger.[15] Furthermore, EGCG is known to increase the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and γ-glutamyl cysteine synthetase (γ-GCS), thereby increasing antioxidant capacity and promoting repair of oxidative damage in the brains of mice.[16,17] Thus, andrographolide and EGCG are potential to be developed as a therapeutic approach in overcoming the risk of nicotine addiction.

Currently, several approved therapies are available to treat addiction to nicotine or tobacco products, including nicotine replacement therapy (NRT), bupropion, and varenicline.[18] However, clinical trials showed that smoking cessation rates range from only 10-20% for NRT, 15-25% for bupropion, and 23-40% for varenicline.[19] Therefore, this topic has attracted academics and researchers to conduct studies both *in vitro* and *in vivo* to develop alternative therapies in overcoming nicotine addiction.[19-21]

Based on the above explanation, the effect of increasing SOD and decreasing ROS in the brain produced by andrographolide and EGCG may inhibit signaling in reward-related behavior in association with nicotine addiction in the dopaminergic pathways of the mesocorticolimbic system. No recent studies evaluated the activity of andrographolide and EGCG in treatingnicotine addiction. This study was conducted to observe the effect of andrographolide and EGCG on the risk of nicotine addiction after administration of nicotine and cigarette smoke extract (CSE), particularly related to the reward effect formed.

Materials and Methods

Experimental animals

Balb/c male mice, with the bodyweight of 20-30 grams and 8-12 weeks old, in healthy status, with normal behavior, and no visible abnormalities in the body were used in this study. The conditions of the cages for mice were regulated by a dark/light cycle, each 12 hours dark and 12 hours light, humidity (60 ± 10%), and temperature (25 ± 2 °C). All mice were acclimatized for seven days before treatment. The treatment protocol of experimental animals were carried out at the Animal Laboratory Research Center at the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia.

Drugs and experimental design

Andrographolide was purchased from Tokyo Chemical Industry (Japan), and EGCG was obtained from Professor Djoko Agus Purwanto (Universitas Airlangga, Indonesia). Andrographolide and EGCG were prepared *recenter paratus*, not more than 30 minutes before use. Nicotine was purchased from Sigma-Aldrich (United States), dissolved in normal saline (0.9% NaCl) from PT. Otsuka Indonesia. Furthermore, the preparation of CSE refers to the protocol developed previously by using a device that bubbles the smoke extract from lit commercial filter cigarettes into normal saline to obtain water-soluble components from cigarette smoke, such as nicotine and minor alkaloids. The cigarette is smoked for 2 seconds followed by 30-second intervals in 35 ml of normal saline. CSE is prepared *recenter paratus* every time it is treated to avoid degradation of the compounds contained in it.[22]

Regarding the experiment, 35 mice were randomly divided into seven groups (n=5) consisting of: (1) the control group injected with normal saline 2 ml/kg BW subcutaneously (s.c.); (2) the nico group injected with nicotine 0.6 mg/kg BW s.c.; (3) the CSE group injected with CSE 1.0 mg/kg BW s.c.; (4) the andro + nico group injected with andrographolide 50 mg/kg BW i.p. and followed by injection of nicotine 0.5 mg/kg BW s.c.; (5) the andro + CSE group injected with andrographolide 50 mg/kg BW i.p. and followed by injection of CSE 1.0 mg/kg BW s.c.; (6) the EGCG + nico group injected with EGCG 50 mg/kg BW i.p. and followed by injection of nicotine 0.5 mg/kg BW s.c.; (7) the EGCG + CSE group injected with EGCG 50 mg/kg BW i.p. and followed by injection of

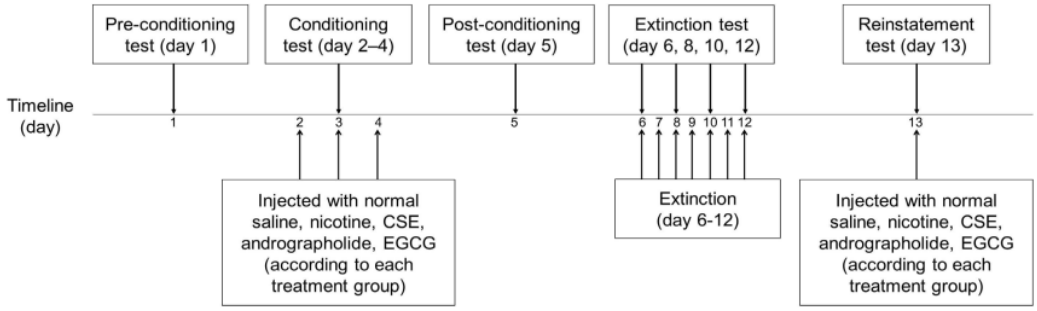

Figure 1. Operational timeline of the conditioned place preference (CPP) method.

CSE 1.0 mg/kg BW s.c. Either andrographolide or EGCG was injected 60 minutes before nicotine or CSE injection. Furthermore, at 10 minutes after injection of nicotine or CSE, mice were tested on a conditioned place preference (CPP) instrument, which consisted of two compartments, namely a black compartment with a smooth floor and a white compartment with a rough bottom. The CPP method with a biased design in which the drug is placed in the least preferred compartment and vice versa, the vehicle is paired with the preferred compartment is used to evaluate the effect of reward or addiction level. This method consists of several stages: preconditioning, conditioning, and postconditioning tests followed by extinction and reinstatement tests (briefly shown in Figure 1).

Pre-conditioning evaluation

The pre-conditioning test was performed on day 1 to determine which compartment was paired with the drug. Mice were placed in the middle between the two compartments and then left for 15 minutes to choose the compartment freely. The duration of time spent by mice in each compartment was recorded. Then, the less preferred compartment is paired with the drug.

Conditioning evaluation

The conditioning test was carried out for three days (days 2-4) twice a day, which was in the morning (09.00 am) and in the afternoon (03.00 pm) with the administration of drugs or normal saline according to the specified schedule. This phase is the stage of acquisition or intoxication in the initial cycle of addiction. On day 2, mice were given normal saline 1 ml/kg BW s.c. in the morning in all groups and paired with the preferred compartment for 30 minutes by being given a closed intercompartment bulkhead. Then, in the afternoon, the mice were given treatment according to their respective treatment groups (group 1 – group 7) as previously described, then put into the less preferred compartment for 30 minutes. On day 3, all mice were treated with a morning and evening shift schedule from the second day. On day 4, all mice were treated with a morning and evening alternation schedule from the third day (given the same treatment as the second day).

Post-conditioning evaluation

The post-conditioning test was performed on day 5 to see an increase in time in the drug-paired compartment, representing an emergent reward effect or acquisition stage. The test procedure is the same as the test procedure for the pre-conditioning test.

Extinction evaluation

The extinction test was carried out on days 6, 8, 10, 12 to observe the occurrence of withdrawal (reduction of reward effect) on nicotine or CSE because the exposure was stopped. At this stage, all mice were not administered with any injection of vehicle or drugs for seven days (days 6-12). The extinction test procedure is the same as the pre-conditioning and post-conditioning tests.

Reinstatement evaluation

The reinstatement test was carried out on day 13 to observe whether there was a tendency to relapse due to nicotine or CSE administration again after extinction. At this stage, mice were administered with normal saline, nicotine, CSE, andrographolide, and EGCG according to their respective treatment groups. After 10 minutes, the mice were put into the CPP instrument for observation of place preference for 15 minutes with the same procedure as the pre-conditioning, post-conditioning, and extinction test procedures.

Data analysis

The normality of data was evaluated using the Kolmogorov-Smirnov test. A parametric one-way analysis of variance (ANOVA) method and continued with Tukey's posthoc test was used when data showed normal distribution. All data obtained were analyzed statistically using Graph-Pad Prism 9.0 software. Data are presented as mean ± SEM values.

Results

Andrographolide and EGCG attenuate nicotine-induced reward effect in mice

In this study, the nicotine-induced reward effect in mice was represented by the CPP score obtained by calculating the difference between the post-conditioning test and

the pre-conditioning test scores. The results obtained showed a significant increase in CPP scores in the nicotine group ($p<0.001$) and the CSE group ($p<0.001$) compared to the control group. The nicotine group which previously received pharmacological treatment with andrographolide ($p<0.001$) or EGCG ($p<0.001$) showed a significantly lower CPP score than the nicotine group without pharmacological treatment. Likewise, the CSE group which received pharmacological treatment with andrographolide ($p<0.001$) or EGCG ($p<0.01$) showed a significantly lower CPP score compared to the CSE group without pharmacological treatment. Overall data regarding the mean CPP scores in each group is shown in Figure 2.

Effect of andrographolide and EGCG on the extinction and relapse of addiction in mice

After the post-conditioning test was carried out, the extinction phase was then monitored. This stage is aimed at observing the tendency to decrease the reward effect of nicotine, which is represented by a decrease in the time spent by mice in the compartment paired with the drug compared to the post-conditioning results. The results obtained showed that in the nicotine group, there was a significant reduction in the extinction phase compared to the post-conditioning phase ($p<0.05$). Meanwhile, in the nicotine group receiving pharmacological treatment with andrographolide ($p>0.9999$) or EGCG ($p=0.7880$), there was no reduction in time in the extinction phase compared to the post-conditioning phase.

The extinction test was followed by a reinstatement test on the next day, which was aimed at observing the potential for nicotine relapse characterized by an increase in the time the mice spent in the drug-paired compartment, compared to the extinction phase. The results obtained showed that in the nicotine group ($p=0.9553$), the CSE group ($p=0.5631$), the nicotine group previously given pharmacological treatment with andrographolide ($p>0.9999$) or EGCG ($p=0.9868$) and the CSE group previously given pharmacological treatment with andrographolide ($p>0.9998$) or EGCG ($p>0.9999$) there was no significant increase in the time spent in the drug-paired compartment of the mice in the reinstatement phase compared to the extinction phase. Overall the data related to the comparison of the results of the post-conditioning test, extinction test, and reinstatement test are shown in Figure 3.

Discussion

Nicotine is the main component in cigarettes that is pharmacologically active and causes addiction.[15] In preclinical research, the majority of nicotine addiction model development mostly uses nicotine induction. However, this model does not describe smoking addiction comprehensively because cigarettes contain various compounds whose role in smoking addiction was not fully explored. CSE is an approach to obtain constituents other than nicotine from cigarettes, especially water-soluble compounds inhaled from cigarette smoke that has the potential for preclinical research using acquisition, reward, and reinforcement models.[22]

The dose of nicotine used in this study was 0.5 mg/kg BW considering that at this dose, nicotine induces a

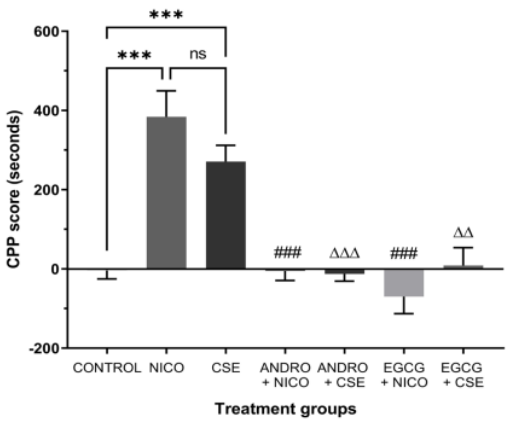

Figure 2. Andrographolide and EGCG attenuate nicotine-induced reward effect in mice. CPP score data is shown as mean value ± SEM. ***p < 0.001 versus CONTROL; ###p < 0.001 versus NICO; ΔΔΔp < 0.001 versus CSE; ΔΔp < 0.01 versus CSE. CONTROL : normal saline 1 ml/kg BW; NICO : nicotine 0.5 mg/kg BW; CSE : CSE 1.0 mg/kg BW; ANDRO + NICO : andrographolide 50 mg/kg BW + nicotine 0.5 mg/kg BW; ANDRO + CSE: andrographolide 50 mg/kg BW + CSE 1.0 mg/kg BW; EGCG + NICO : EGCG 50 mg/kg BW + nicotine 0.5 mg/kg BW; EGCG + CSE : EGCG 50 mg/kg BW + CSE 1.0 mg/kg BW.

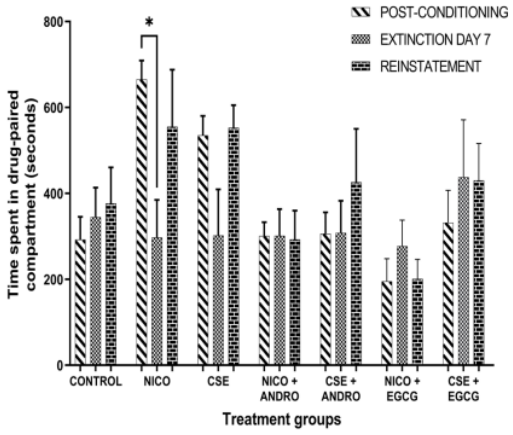

Figure 3. Effect of andrographolide and EGCG on the extinction and relapse of addiction in mice. Data regarding the time the mice spent in the drug-paired compartment were shown as mean ± SEM . values. *p < 0.05 versus post-conditioning (in NICO group). CONTROL : normal saline 1 ml/kg BW; NICO : nicotine 0.5 mg/kg BW; CSE : CSE 1.0 mg/kg BW; ANDRO + NICO : andrographolide 50 mg/kg BW + nicotine 0.5 mg/kg BW; ANDRO + CSE: andrographolide 50 mg/kg BW + CSE 1.0 mg/kg BW; EGCG + NICO : EGCG 50 mg/kg BW + nicotine 0.5 mg/kg BW; EGCG + CSE : EGCG 50 mg/kg BW + CSE 1.0 mg/kg BW.

reward effect.[23,24] Meanwhile, the administered dose of CSE is twice the nicotine dose, which is 1.0 mg/kg BW because at that dose, it has been optimized and produces a reward effect equivalent to 0.5 mg/kg BW nicotine. With these doses, when converted to adult human subjects (weighing 70 kg) the administered dose was equivalent to nicotine 35 mg/day and CSE 70 mg/day. Based on clinical studies, daily intake of nicotine averaged 37.6 (±17.7) mg but varied widely among subjects (10.5 to 78.6 mg).[23] Thus, the nicotine and CSE doses administered in this study were in line with the nicotine doses consumed by smokers.

However, the nicotine concentration in CSE used in this study was not evaluated, thus opening the opportunity for further exploration regarding whether the nicotine concentration in the CSE group at a dose of 1.0 mg/kg BW was comparable to the nicotine concentration in the 0.5 mg/kg BW group or not. Further exploration is also important to determine whether the resulting reward effect is dominated by the effects of nicotine or is also influenced by various other compounds contained in CSE. Both nicotine and CSE were administered to mice by the subcutaneous route because based on previous studies, the use of this route provided a stronger and more consistent addictive effect than the intravenous route.[13] Furthermore, the dose of andrographolide and EGCG used was 50 mg/kg BW intraperitoneally. The dose and route of administration chosen were based on a previous study which revealed that the administration of andrographolide 50 mg/kg BW i.p. decreased tissue MDA levels and increased SOD levels in the rat brain.[25] Then, the administration of EGCG 50 mg/kg BW i.p. was also shown to provide endogenous protection against hepatic ischemia-reperfusion injury in mice due to increased ROS.[24,26] Meanwhile, the schedule of andrographolide and EGCG administrations refers to the schedule of nicotine and CSE administrations.

The first stage in the addiction cycle is acquisition. In the CPP method, this stage is represented by the CPP score. The findings obtained in this study indicate that the test group injected with nicotine or CSE has been shown to provide a reward effect that contributes to an increased risk of addiction. This was confirmed by the CPP score of the nicotine or CSE group, which was significantly higher than that of the control group (injected with normal saline). This study is also in line with previous findings, which suggested that subcutaneous administration of nicotine 0.5 mg/kg BW resulted in a significant preference for the drug-paired compartment.[24] The rewarding effect may arise because nicotine stimulates nerves to interact with the basal forebrain, particularly the nucleus accumbens (NAc). Furthermore, there is an increase in dopamine, serotonin, and other neurotransmitters that activate the mesolimbic and mesocorticolimbic dopamine systems, strengthening the positive effects of nicotine.[27]

Based on the results of previous studies, the administration of CSE provides a more significant reward effect than the administration of nicotine because CSE contains ingredients other than nicotine which function to inhibit monoamine oxidase (MAO), which increases dopaminergic and serotonergic neurotransmitters that contribute to self-administration or addiction.[28-30] Meanwhile, a more significant reward effect could also be associated with higher nicotine concentrations in the CSE group than in the nicotine group. However, the average CPP score between the test groups receiving nicotine and CSE was not significantly different in this study. This finding may be caused by a lot of smoke comes out of the device so that the content of nicotine and other substances are reduced during the smoking process. These conditions may result in a low concentration of nicotine in the CSE. Furthermore, this condition is also described in a review which states that CSE has limitations, e.g. the levels produced vary according to the tool settings and environmental parameters.[30]

The nicotine or CSE group previously given pharmacological treatment with andrographolide or EGCG did not experience a significant increase in CPP scores compared to the control group, but each was significantly different compared to the nicotine group and the CSE group. The above findings indicate that the administration of andrographolide and EGCG attenuates the reward effect induced by nicotine or CSE, resulting in a reduced risk of addiction. In addition to nicotine stimulation of the NAc, the reward effect is also strengthened as a result of the toxic effects of cigarette smoke on the brain due to increased ROS production, which results in the destruction of the endogenous antioxidant defense system in the NAc, so that the response of nAChRs to nicotine will increase.[10] Antioxidants andrographolide and EGCG are thought to reduce or even prevent this mechanism by attracting ROS through their polyphenol hydrogen groups. Furthermore, both andrographolide and EGCG were shown to reduce inflammation-mediated dopaminergic neurodegeneration in mesenphalic neuron-glia by inhibiting microglial activation.[31,32] Microglial activation results in the induction of pro-inflammatory signaling to the nucleus accumbens which is a central location in the nicotine addiction process.[33] Overall, in addition to their potential antioxidant action, andrographolide and EGCG may reduce the risk of nicotine addiction through microglial inhibition mechanisms and reduce inflammation-mediated dopaminergic neurodegeneration.

The second stage in the addiction cycle is the withdrawal effect. In the CPP method, this stage is represented by the extinction phase. In this phase, an extinction test is conducted to determine the level of strength of nicotine or CSE and how long the drug-seeking behavior lasts when nicotine or CSE is discontinued.[9] The findings obtained indicate that the test group experiencing extinction is the nicotine group, with the average time spent in the drug-paired compartment during the extinction test experiencing a significant decrease compared to the post-conditioning test. Several studies confirm that the reward effect resulting from previous drug exposure has been reduced when reaching extinction, and causes a

withdrawal effect.[27,34,35] This withdrawal effect occurs because the dopamine function of the reward effect decreases and stress neurotransmitters in the brain, such as corticotropin and dynorphin, increase in the extended amygdala nerves, resulting in negative responses such as fatigue and decreased mood.[9,34]

The CSE group also tended to experience decreased mean time spent in the drug-paired compartment during the extinction test compared to the post-conditioning test, but not significantly. In CSE, there are constituents involved in the MAO inhibitory mechanism which causes the accumulation of dopamine and serotonin levels in the Nac resulting in a more robust addiction response so that the reward effect lasts longer than the reward effect produced by nicotine administration.[28-30] Meanwhile, in the control treatment group, as well as the nicotine or CSE group, which had previously been administered with andrographolide or EGCG treatment, all three did not show extinction. This finding is possible because the administration of andrographolide or EGCG reduces and prevents the reward effect so that when nicotine or CSE is discontinued, it does not result in a decrease in the reward effect that causes a withdrawal effect.[36]

The last stage of the addiction cycle is the reinstatement (preoccupation) stage, which is the stage where relapse occurs and shows drug-seeking behavior after drug withdrawal.[33] In the CPP method, this stage is represented through a reinstatement test.[9] Reinstatement or relapse was indicated by the average time spent in the drug-paired compartment during the reinstatement test increased compared to the 7th day of extinction test.[28] Inductions that cause relapse include drug induction, which in this study is nicotine or CSE induction, and stress induction. The findings showed that the nicotine and CSE-treated groups both tended to experience an increase in the mean time spent in the drug-paired compartment during the reinstatement test compared to the 7th day of extinction, but this was not statistically significant. Reinstatement failure from prior nicotine administration was also found, where nicotine administration alone did not cause relapse upon re-administration but succeeded in causing relapse in rats administered before nicotine administration. This happens for several reasons, such as dose differences, time intervals between drug administration and testing, route of drug administration, and administration of stimuli that play an essential role in increasing the response to reinforcement.[37,38]

Furthermore, there was no relapse in the CSE group, which may be because the CSE group has not experienced extinction. Relapse or reinforcement may occur when the experimental animal has experienced extinction and has shown drug-seeking behavior.[34] An extinction test period of more than seven days may be required to observe relapse in the CSE group. As for the nicotine or CSE group previously administered andrographolide or EGCG treatment, there was no relapse, as evidenced by the absence of an increase in time spent in the drug-paired compartment during the reinstatement test compared to the extinction test. Experimental animals administered with andrographolide or EGCG treatment before being injected with nicotine or CSE did not experience relapse, as was also found in the control group. This is because these groups did not previously experience the reward effect in the acquisition phase and did not experience drug-seeking in the extinction phase.

Taken all together, this is the first study that has successfully explored the potential of andrographolide and EGCG in reducing the risk of nicotine addiction. Based on the findings of this study, there are still limitations, challenges, and opportunities for further studies. There are limitations related to the lack of knowledge of nicotine concentrations in CSE used in this study. Therefore, to strengthen the findings obtained, it is necessary to conduct further research related to the analysis of nicotine concentrations in CSE. In this study, there are also challenges and opportunities in the future, that is to investigate the underlying mechanisms or signaling pathways related to the potential efficacy of andrographolide and EGCG in reducing the risk of nicotine addiction (e.g. oxidative stress, microglial inhibition, and inflammation-mediated neurodegeneration pathways). In addition, further developments may be carried out i.e. developing the non-invasive dosage forms (e.g. oral dosage forms) that are ideal as a preventive therapeutic approach to overcome nicotine addiction. However, the administration of andrographolide and EGCG at a dose of 50 mg/kg BW i.p. has been shown to reduce the risk of nicotine addiction too high when converted to an oral dosage form. Thus, for further development, optimizations should be carried out using dose variations to obtain the minimum dose needed to produce a nicotine addiction risk-reduction effect. Evidently, the findings of our present studies serve as the basis for further development.

Conclusion

In summary, the findings indicate that andrographolide and EGCG may lower the risk of addiction and are potential to be developed as a preventive therapy for smoking cessation. Further research to study the mechanism of action of andrographolide and EGCG in lowering the risk of nicotine addiction and clinical trials need to be conducted to support the findings of this study.

Ethical Issues

All protocol in this study was prepared based on the Guideline for the Care and Use of Laboratory Animals issued by the National Institutes of Health revised in 1985 and approved by the research ethics committee of the Faculty of Veterinary Medicine Universitas Airlangga (Animal Care and Use Committee) with a certificate of ethical clearance No. 2.KE.064.06.2021.

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Author Contributions

SS validated and monitored the experimental study. KI validated the experimental study and collected the relevant data. ADN drafted and revised the manuscript and conducted formal analysis of the data. VS and NRP collected the relevant data and visualized the collected data. CA created the methodology of the study and revised the manuscript. YY created the concept and provided the resources of the study. MR created the concept and the methodology of the study and obtained the funding of the study. All authors read and approved the final manuscript.

Conflict of Interest

The authors report no conflicts of interest.

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