Pharmacoscript
Haq et al./Pharmacoscript, Volume 9, No, 1, Februari 2026, 285-296
P-ISSN : 2622-4941 | E-ISSN : 2685-1121

THE EFFECTS OF ARABICA COFFEE (*Coffea arabica*) FRUIT SKIN FERMENTED WITH KOMBUCHA ON NICOTINE-DEPENDENT MICE

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Received: 25/04/2026 Accepted: 27/04/2026 Published: 29/04/2026

ABSTRACT

Nicotine is an addictive substance in cigarettes that causes dependence, making it difficult for smokers to quit. Treatment for nicotine dependence has limitations in terms of low effectiveness and side effects. Alternative treatments based on natural ingredients, such as coffee fruit skin or cascara (CA) which is rich in polyphenols, flavonoids, caffeine, and chlorogenic acid, offer neuroprotective potential to reduce nicotine dependence with minimal side effects. Fermentation using kombucha (KO) can increase the content of these bioactive compounds. This study aims to evaluate the effect of cascara fermented with kombucha on mice experiencing nicotine dependence. The study used a Conditioned Place Preference (CPP) design in male Swiss Webster mice induced by nicotine at a dose of 0.5 mg/kg BW. The test animals were given cascara and cascara-kombucha preparations at the same dose of 0.65 mL/20 g BW orally. In the pre-test stage, the initial residence time of the mice in each compartment was measured for baseline preference. The conditioning phase was conducted by administering test preparations and nicotine to selected compartments. The post-test measured the residence time in the nicotine compartment for 15 minutes. The CPP score was calculated as the difference between the post-test and pre-test residence times. Cascara and cascara-kombucha produced lower CPP scores than the nicotine group. Cascara-kombucha showed a better effect with a lower CPP score than cascara alone. Fermented cascara kombucha has suggested potential as an alternative therapy to overcome nicotine dependence.
Keywords: Cascara, Kombucha, Nicotine Dependence, Conditioned Place Preference (CPP)

INTRODUCTION

Smoking is a health problem associated with various diseases, such as lung disease, cardiovascular system disease, and reproductive issues (Varghese & Muntode Gharde, 2023). Long-term cigarette use can also increase the risk of mortality (WHO, 2014). Tobacco users are spread across the globe, reaching more than 1 billion people, with a prevalence of approximately 20.9% in 2025 (ASH, 2019; WHO, 2025). In 2030, it is estimated that more than 8 million people will die annually due to tobacco use (Sakthisankaran et al., 2024). In 2024, the prevalence of smoking in Indonesia was quite high compared to the global average, standing at 28.99% (BPS, 2024).

The nicotine contained in cigarettes makes it difficult for individuals to quit smoking. This is because nicotine affects the brain's reward system, which can produce relaxing, calming, and euphoric effects (Picciotto & Kenny, 2013). Furthermore, nicotine can trigger oxidative stress and inflammation in brain tissue, leading to structural brain damage, which is also linked to dependence (Mohamed et al., 2022). Conventional therapies to address nicotine dependence include nicotine replacement therapy, bupropion, varenicline, and acetylcysteine (AC), which is currently in the developmental stage. These treatments still show limited effectiveness and have been proven to cause various side effects such as headaches, nausea, vomiting, digestive disorders, insomnia, skin irritation, palpitations, and chest pain (Mills et al., 2010; Safitri et al., 2021). Therefore, the development of alternative treatments derived from natural ingredients has become crucial.

One potential source is Arabica coffee cherry husk (cascara) waste and kombucha, which have not been optimally utilized; these are products rich in beneficial compounds, including antioxidants (Júnior et al., 2022). Cascara contains various active compounds such as alkaloids, flavonoids, and terpenoids, which exhibit significant antioxidant activity (Sales et al., 2023a). The antioxidant activity of the husk has been proven effective in reducing oxidative stress, which is often associated with addiction and withdrawal symptoms (Cahyani et al., 2021). Studies indicate that chlorogenic acid and caffeine, the active compounds in cascara, have neuroprotective effects that potentially aid recovery from nicotine addiction by maintaining brain health (Kolahdouzan & Hamadeh, 2017; Mikami & Yamazawa, 2015). In previous research, chlorogenic acid has been shown to reduce nicotine dependence in mice (Lee et al., 2024). Additionally, kombucha, a fermented beverage product, is proven to contain numerous active compounds, including polyphenols, which serve as antioxidants capable of reducing reactive oxygen species (ROS) and oxidative stress—levels of which can increase due to nicotine exposure (Kabiri & Setorki, 2016; Sales et al., 2023). To date, no research has been conducted to test the effects of kombucha-fermented coffee cherry husk on animals experiencing nicotine dependence. Thus, this study is necessary to provide scientific evidence regarding this potential.

METHODS

Materials

Coffee fruit skin (cascara) taken from the Merapi mountains of DIY, nicotine dihydrogen tartrate from Sigma Aldrich, acetylcysteine, SCOBY (Borneo Kombucha), 70% ethanol, 96% ethanol, distilled water, granulated sugar, toluene, hydrochloric acid, chloroform, ethanol, magnesium, 10% vanillin, 1% FeCl3, Dragendorf reagent, Mayer reagent, Liebarman-Bauchardat reagent, Mayer reagent, amyl alcohol, 1% gelatin, ether, 5% potassium hydroxide.

Male Swiss Webster mice were obtained from the Bandung Institute of Technology (ITB) research center, aged 6-8 weeks and weighing 20-25 grams. Animals were randomly selected, acclimatized for 14 days before treatment, and given free access to food and water. Animal care and treatment procedures were approved by the Preclinical Ethics Committee of the Faculty of Pharmacy, Jenderal Achmad Yani University, under Number: 11031/KEP-UNJANI/III/2025.

The Conditioned Place Preference apparatus consists of two main compartments in the form of square boxes (25 x 25 x 30 cm) and one middle compartment (10 x 10 x 30) with a guillotine door in the middle. Each compartment has different conditions. Box 1 has smooth black walls with a smooth black floor, while box 2 has white walls with a rough black floor and a gray middle compartment.

Research Path

1. **Sample Collection and Preparation**

Cascara was obtained from a coffee plantation in the Merapi Mountains of Yogyakarta, dried in the sun for three days. Determination was carried out at the Plant Taxonomy Laboratory of the Faculty of Mathematics and Natural Sciences, Department of Biology, Padjadjaran University (UNPAD). The animals test used were Swiss Webster mice obtained from the School of Life Sciences and Technology, Bandung Institute of Technology. Male, 6-8 weeks old, and weighing 20-25 g. Animals were randomly selected, marked for identification, and acclimatized for 5-7 days before treatment.

2. **Extraction and Phytochemical Screening**

Cascara was placed in a beaker glass and distilled water was added up to 250 mL, then heated at 90°C for 15 minutes. Then, it was filtered and the filtrate was obtained. Phytochemical screening was carried out to identify the content of secondary metabolites in cascara simplicia, cascara decoction, and fermented cascara kombucha, including testing for flavonoids, alkaloids, saponins, tannins, polyphenols, Monoterpenoid-Sesquiterpenoids, and steroids/triterpenoids.

3. Cascara-Kombucha Preparation

Cascara was placed in a beaker glass and distilled water was added up to 250 mL, then heated at 90°C for 15 minutes. Then, it was filtered and the filtrate was obtained. Phytochemical screening was carried out to identify the content of secondary metabolites in cascara simplicia, cascara decoction, and fermented cascara kombucha, including testing for flavonoids, alkaloids, saponins, tannins, polyphenols, Monoterpenoid-Sesquiterpenoids, and steroids/triterpenoids.

4. Experimental

This study received approval from the Animal Pre-Clinical Ethics Committee of the Faculty of Pharmacy, UNJANI (No: 11031/KEP-UNJANI/III/2025). Twenty-five male Swiss Webster mice were used and randomly divided into five groups of five. Prior to treatment, the mice underwent a 14-day acclimatization period with free access to standard pellets and water. Consisting of a comparison group that was given N-Acetylcysteine (26 mg/kg BB) and nicotine (0.5 mg/kg BB), the normal group was given 0.5% CMC-Na suspension and 0.9% NaCl solution (0.5 ml/20 g BB), the control group was given 0.5% CMC-Na suspension and nicotine (0.5 mg/kg BB), test group I was given cascara (0.65 ml/20 g BB) and nicotine (0.5 mg/kg BB), test group II was given cascara-kombucha (0.65 ml/20 g BB) and nicotine (0.5 mg/kg BB).

4.1 Handling Habituation

On days 1 through 3, handling habituation was performed in the testing room. Mice were picked up using gloves cleaned with 70% ethanol and placed on the hand. They were allowed to freely explore the arm for 3 minutes, then placed on the CPP instrument to explore all compartments for 3 minutes.

4.2 Pre Test

On the 4th day, a pre-test was conducted. The mice were placed in the center of the CPP compartment. They were allowed to explore the CPP apparatus for 15 minutes, then recorded the time the mice occupied the selected compartment.

4.3 Conditioning Test

On days 5 to 8, a conditioning test was conducted to condition the mice to prefer the compartment containing nicotine. Mice were given 0.9% NaCl in the morning, then kept in the selected compartment for 30 minutes, then transferred to their cage. After 4 hours, the mice were given nicotine and kept in the selected compartment for another 30 minutes.

4.4 Post Test

On day 9, a post-test was conducted. Mice were placed in the center of the CPP compartment to explore the CPP apparatus for 15 minutes. The time the mice spent in their chosen compartment was then recorded. The length of time the mice spent in their chosen compartment can be used to assess nicotine dependence in the mice.

Data analysis

The obtained duration parameters were statistically analyzed using a One-Way ANOVA test assisted by the GraphPad program. Statistical data were used to assess the significance ($p<0.05$) of the test substance's effect on the control group. The test substance that had an effect could reduce the length of time the mice occupied the chamber containing nicotine.

RESULTS AND DISCUSSION

Organoleptic macroscopic testing was conducted to determine the quality of simplisia used including aroma, color, taste, texture and shape as shown in table 1. Based on the results of organoleptic identification and determination, it is known that the sample used is an Arabica coffee plant (Coffea arabica) which has good quality. Cascara Coffea arabica has a distinctive shape and color, as well as a fairly thick surface, thus protecting the fruit from pests and insect.

Table 1. Organoleptic evaluation

Examination Parameters	Examination Results
Odor	Characteristic odor
Color	Blackish Brown
Taste	Bitter
Texture	Fine
Form	Powder

In addition, the testing of the characteristics of the simplicia was carried out to assess the quality of the simplicia materials used including water content, water-soluble extract content, ethanol-soluble extract content, total ash content, water-soluble ash content and acid-insoluble ash content. The results of the characteristic testing are shown in table 2. The simplicia used showed good quality because it met the requirements for testing the characteristics of the simplicia, especially water content and acid-insoluble ash content.

Table 2. Characterization of cascara simplisia

Examination Parameters	Examination Results
Water content (%v/w)	9,2±0,6
Water-soluble extractive value (%w/w)	23±0,9
Ethanol-soluble extractive value (%w/w)	14±0,7
Total ash content (%w/w)	11±0,7
Water-soluble ash content (%w/w)	12±0,4
Acid-insoluble ash content (%w/w)	2±0,4

Phytochemical screening was conducted to identify secondary metabolite compounds that have the potential to have pharmacological activity. Tests were conducted on simple materials, extracts, and fermented cascara extracts. The results can be seen in Table 3. Phytochemical screening (Pradnyasuari & Putra, 2023) showed that cascara simple materials contain secondary metabolites in the form of alkaloids, flavonoids, polyphenols, saponins, monoterpenes-sesquiterpenes, and steroids/triterpenoids. The extract contains secondary metabolites in the form of alkaloids, flavonoids, polyphenols, saponins, and steroids/triterpenoids, while kombucha contains secondary metabolites in the form of alkaloids, flavonoids, quinones, and steroids/triterpenoids. Through phytochemical screening, researchers can gain a clearer picture of the potential use of plants for the development of new drugs or nutraceuticals, a key requirement in modern medicine (Pradnyasuari & Putra, 2023). Saponin content was not detected in cascara-kombucha, indicating the possible influence of fermentation on reducing saponin levels (Arjmand et al., 2023). Quinones showed positive results in cascara-kombucha indicating that the fermentation process can increase the formation and extraction of secondary metabolite compounds including quinones (Sales et al., 2023).

Table 3. Phytochemical screening

Compound Groups	Cascara Simplisia	Cascara Extract	Kombucha-Fermented Cascara
Alkaloids	+	+	+
Flavonoids	+	+	+
Polyphenols	+	+	+
Tannins	-	-	-
Saponins	+	+	-
Quinones	-	-	+
Monoterpenoids-Sesquiterpenoids	+	-	-
Steroids-Triterpenoids	+	+	+

The success of nicotine induction in mice is very important for nicotine dependence. The results of nicotine induction and residence time in the nicotine and saline groups are relatively constant as shown in Figure 1. In the CPP test, nicotine induction of mice confirmed the persistence of nicotine dependence in the test animals. The residence time of animals given nicotine increased, while that of animals given saline remained relatively constant. These results align with several previous studies showing that nicotine dependence induction using the conditioned place preference (CPP) method can be achieved in subjects receiving nicotine at a dose of 0.5 mg/kg. (Adeniyi et al., 2020; Anggadiredja et al., 2011; Yunusoğlu, 2023).

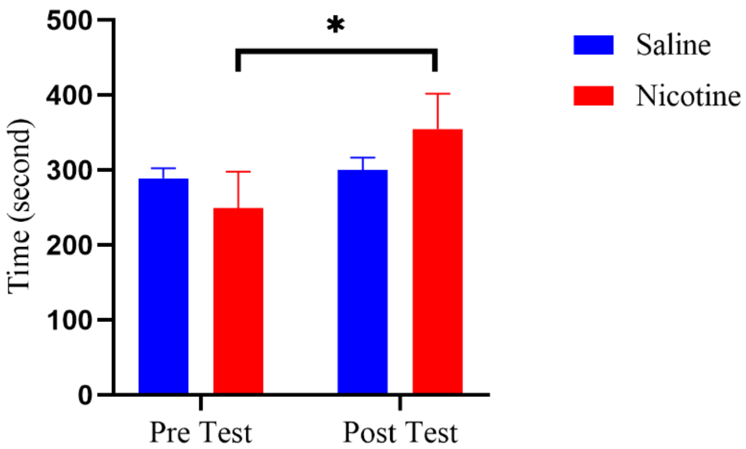

Figure 1. Nicotine Induction. * = Significant Difference ($p<0.05$)

The results of the nicotine dependence test can be seen in Figure 2, which shows the test material has a lower CPP score than the nicotine group. The results of the study showed that administering cascara and cascara kombucha can reduce the development of nicotine dependence, as indicated by a lower CPP score than the nicotine group, although the difference was not significant and the results were not better than mice given acetylcysteine. Administration of cascara kombucha showed better results than cascara in the development of nicotine dependence, this indicates that the potential for cascara increases when fermented with kombucha.

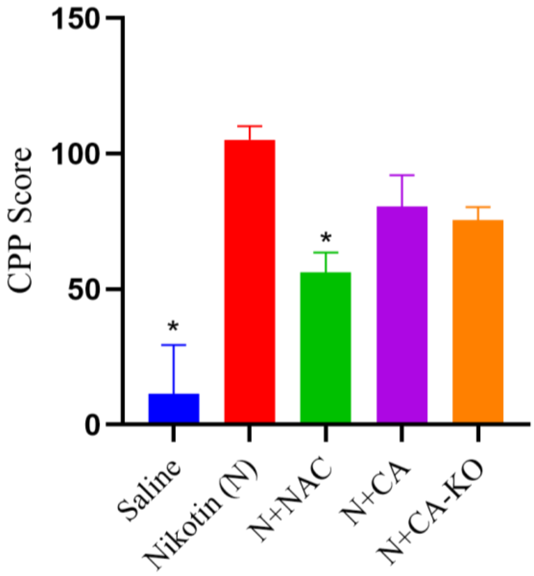

Figure 2. Nicotine dependence test.

* = Significantly different from the Nicotine group ($p<0.05$)

Neuroprotective activity is related to the ability to reduce the risk of neurodegenerative diseases such as Alzheimer's, Parkinson's, ischemic stroke and also dependence on substances such as nicotine, morphine and alcohol. (He et al., 2023; Mohamed et al., 2022). Antioxidant activity is related to the ability to ward off free radicals that occur in various diseases including conditions of oxidative imbalance in nicotine dependence (Caliri et al., 2021; Mohamed et al., 2022).

Research by Lee HJ et al. which discusses the effects of extracts and components of acanthopanax koreanum on animals experiencing nicotine dependence and nicotine withdrawal symptoms using the CPP method, in the study it was stated that chlorogenic acid isolates derived from acanthopanax koreanum can reduce nicotine dependence by normalizing dopamine concentrations and nicotinic receptor expression levels. (Lee et al., 2024). Chlorogenic acid is also an active compound found in cascara which may play a role in providing the effect of reducing nicotine dependence. (Machado et al., 2022). Then the research results are also supported by research from Sales AL et al. which discusses the comparison of the increase in bioactive compounds in cascara kombucha and black tea kombucha, in the study it is stated that cascara and black tea fermented using kombucha can increase compounds including phenolics including chlorogenic acid (Sales et al., 2023).

CONCLUSIONS

Cascara kombucha has the potential to reduce the effects of nicotine dependence based on a conditioned place preference paradigm in a mouse model. These results could form the basis for developing nicotine dependence therapy in smokers.

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