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The cessation and detoxification effect of tea filters on cigarette smoke

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To treat tobacco addiction, a tea filter was developed and studied for smoking cessation. This work reports the smoking cessation effect of tea when it was used as a component of cigarette filters. In one trial it was found that after using the tea filters for 2 months, the volunteer smokers decreased their cigarette consumption by 56.5%, and 31.7% of them stopped smoking. This work identified a new method and material, tea filter and theanine, which inhibit tobacco and nicotine addiction and provide an effective strategy for treating tobacco addiction.

cigarette smoking addiction, cigarette cessation, tea filter, theanine, nicotine dependence, nicotine acetylcholine receptor (nAChR), dopamine, public health, free radical

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Cigarette smoking has been linked to many life threatening diseases including heart disease, cancer and chronic obstructive pulmonary disease [1–5]. There are about 1.25 billion smokers in the world and 5 million die every year because of smoking-related diseases [6], exceeding many other diseases combined. It is estimated that the global cost for smoking-related disease is about 1.2 trillion yuan each year, resulting in one of the world's largest public human health problems. Many methods have been developed for smoking cessation by researchers and clinicians [7–14]. However, due to the addictive nature of nicotine, quitting smoking remains extremely difficult. Despite all efforts, currently available smoking cessation methods produce only modestly successful rates with frequent relapse. In addition, they are often perceived as being inconvenient and lead to a wide variety of side effects [7–13]. Therefore, the need for developing alternative remains a high public health priority, smoking cessation strategies with improved efficacy and fewer side effects.

Epidemiological and experimental evidence suggests that drinking tea is adversely associated with cancer [15], hyperglycemia [16] and other diseases [17]. In addition, our work has shown that tea components protect cells from cigarette smoke-induced toxicity [18–23]. We developed a cigarette filter containing tea. Human tests showed that smoking using tea filters significantly decreased the cigarette consumption of the volunteer smokers without any apparent side effects. Further study showed that an amino

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acid derivative of tea, theanine, exerted an effect similar to the nicotine acetylcholine receptor inhibitor.

1 Materials and methods

1.1 Preparation of cigarettes with tea filters

"Honghe" cigarettes purchased from a market (made by the Honghe Tobacco Ltd Company, Yunnan, China) with regular filters (cellulose acetate filter 2.5 cm) were used as the control cigarettes. A complex tea filter consisting of half of a tea filter (1.25 cm) and half of a regular filter (1.25 cm) was used in the human test for the tea filter group. The tea filter half was attached to the cigarette and the cellulose acetate filter half was attached to the tea filter. The total length of the complex tea filter was 2.5 cm, with a similar appearance to a regular cellulose acetate filter (2.5 cm). The theanine in the tea filter was about 1%.

1.2 Human test

A human test was approved by the authorized committee and conducted in the Addiction Branch, Beijing Military Region General Hospital (ClinicalTrials.govIdentifier: NCT00971529). In one trial, one hundred healthy male cigarette smokers, aged 18 to 30 years, were screened using the standard exclusion/inclusion criteria [11]. Thirty of the volunteers were excluded and 70 of them were double-blinded, placebo-controlled and randomized into 2 groups (smoking with tea filters or regular filters). In another trial, 70 healthy male cigarette smokers, aged 30 to 65 years, were screened and 59 volunteers with a longer smoking history and a stronger desire to quit smoking were tested using the tea filter for 3 months. Smoking history, including assessment of nicotine dependence, was evaluated at the screening visit.

Subjects received brief smoking cessation counseling (up to 10 min) at the baseline visit and at each visit afterwards. Self-reported smoking status since the last visit and exhaled carbon monoxide measurement were assessed at each weekly visit. Vital signs, weight, and adverse event information were collected at each visit. Physical examinations were performed prior to randomization and at the final visit. During the follow-up period, use of nicotine replacement therapy did not disqualify subjects from being considered abstinent. Subjects who withdrew or were lost to follow-up were assumed to be smokers for the remainder of the study. The exhaled carbon monoxide was detected by a carbon monoxidemeter (1209-1 CO, DWYER, USA). Cotinine in urine was analyzed using a HPLC (Hitachi, Japan) as reported in the literature [24].

1.3 Animal treatment

Animal experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee. Six to 8 week old female C57BL/6J mice were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Science and housed in a specific parasite free (SPF) environment at 22°C with a 12 h light-dark cycle. Food (Mouse Diet, Beijing Experiment Animal Center) and water were available *ad libitum*. Chemicals were dissolved in saline and subcutaneously injected. Animals were randomly divided into the following groups (n=10) for treatment with different chemicals: Control: mice were injected with 0.9% physiological saline, s.c.; Nicotine: Mice were injected with nicotine (0.5 mg kg^{-1} d^{-1} , s.c.); Nicotine+GTP: mice were lavaged with green tea polyphenols (250 mg kg^{-1} d^{-1}) and injected with nicotine; Nicotine+CF: mice were injected with caffeine (2 mg kg^{-1} d^{-1}, s.c.) and nicotine; Nicotine+TH1: mice were injected with L-theanine-low (250 mg kg^{-1} d^{-1}, s.c.) and nicotine; Nicotine+TH2: mice were injected with L-theanine-high (500 mg kg^{-1} d^{-1}, s.c.) and nicotine; Nicotine+DHβE: mice were injected with Dihydro-β-erythroidine hydrobromide (DHβE) (2.0 mg kg^{-1} d^{-1}, s.c.) and nicotine [25]. Mice were daily injected with nicotine (0.5 mg kg^{-1} d^{-1}) or physiological saline for 1 week. Different compounds were administered 15 min before nicotine injection.

1.4 Conditioned place preference (CPP) test

CPP is a behavioral test and a measure of nicotine reinforcement, that is typically used to study the rewarding properties of nicotine and other drugs [26–28]. This procedure consisted of 3 phases: preconditioning, conditioning, and post conditioning. In our procedure, days 1 and 2 were preconditioning days and mice were allowed to roam freely among the 2 identically sized compartments (20 cm×15 cm×15 cm), which were separated by a narrow compartment (10 cm×15 cm×15 cm), for 900 s and the time spent in each compartment was recorded. According to the test data, mice were divided into 7 groups so that all groups showing an unconditioned preference (n=10 mice per group). On days 3–9, the mice were injected s.c. with saline or chemicals and immediately placed in one of the pairing compartments for 30 min. Five hours later, the mice were injected with the alternate chemicals or saline and immediately placed in the opposite chamber for 30 min. Control groups received saline on both sides of the chamber. On day 10, the animals once again were allowed to freely roam among the 3 compartments for 900 s and the time spent on each side was recorded. The animals were drug-free on preconditioning days and the post conditioning day. The mice were killed by cervical dislocation, and the brains were quickly removed and treated in different ways for analysis.

1.5 Western blotting assay

The mice brain tissues were homogenized on ice in a buffer

(50 mmol L^{-1} Tris-Cl, 150 mmol L^{-1} NaCl, 0.02 NaN_2, 100 μg mL^{-1} PMSF, 1 μg mL^{-1} Aprotitin and 1% Triton X-100) at 0°C for 30 min. Tissue homogenates were centrifuged at 12000×*g* for 25 min at 4°C. Supernatants were collected and analyzed by Western blotting using the standard protocol. Band intensities were quantified using an image analyzing software (NIH Image). Antibodies of AChRα_4 (sc-74519), AChRβ_2 (sc-11372), AChRα_7 (sc-11372) and β-Actin (sc-1616-R) were used to detect the expression of the proteins.

1.6 Dopamine measured by high-performance liquid chromatography (HPLC) with electrochemical detection

Thirty min after subcutaneous injection of saline or different chemicals, mice were killed by cervical vertebra dislocation and both sides of the striatum were carefully isolated. Samples were weighed and homogenized in 1 mL 0.2 mol L^{-1} perchloric acid. Tissue homogenates were centrifuged at 20000 r min^{-1} for 15 min at 4°C and the supernatants were collected for further analysis. The level of the striatal dopamine (DA) was determined using a previously described method by the HPLC ESA-5600A Coularray system (ESA, USA).

1.7 Exposure of rodents to cigarette smoke

To study acute and chronic toxicity, the rodents were exposed to cigarette smoke in a polyacryl glass chamber (35.6 cm×35 cm×20 cm) with two 1.5 cm^2 holes, one located on top of the chamber for ventilation and the other at the bottom as entrance for the gas phase [29]. The gas phase of smoke was delivered with a special pump to the chamber containing one group of 10 rodents. Rodents in group 3 served as a control and were not treated with cigarette smoke.

During the experiments pO_2, pNO_x and pCO_2 were monitored and it was found that the changes of pO_2 and pCO_2 were less than 5%; the level of pNO_x was increased from 0 to about 60 ppm. All of these changes did not reach lethal levels. These results indicated that the differences at death between the different groups were not caused by the changes of pO_2, pNO_x and pCO_2.

1.8 Acute toxicity of cigarette smoke in mice

Twenty four rodents were randomly divided into 3 groups. Rodents in group 1 were treated with the gas phase of smoke from cigarettes with standard filters. Rodents in group 2 were treated with the gas phase of smoke from cigarettes with tea filters. Rodents in group 3 served as a control and were not treated with cigarette smoke. All mice (n=8) were exposed to the gas phase of cigarette smoke as described above, recording the time and number of cigarettes until the lethal endpoint was reached. The deceased mice were examined for histopathological changes and the congestion and haemorrhage in lung tissue were quantified as markers of damage.

1.9 Chronic toxicity of cigarette smoke in rats

Three groups of rats were exposed to the gas phase of cigarette smoke as described above. Each group of 5 rats was exposed to the gas phase of 7 cigarettes during 30 min. This procedure was carried out twice a day, with an intermission of 4 h, over a total time period of 75 d. This protocol was approved by the Animal Experimentation Committee of the China Academy of Traditional Chinese Medicine. Mutagenicity of chronic smoking was established by micronucleus assay. Bone marrow of the sacrificed rats was flushed out of femurs, homogeneously mixed with an equal volume of fetal bovine serum, centrifuged, resuspended and spread on a slide. The smear was air-dried and stained with May-Grünwald/Giemsa. 1000 polychromatic erythrocytes (PCEs) were analyzed per animal for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes (NCE) was determined in the same sample and expressed in NCE per 1000 PCEs.

Specimens from lungs, heart, liver, spleen, kidneys and adrenal glands were taken from organs of all rats in the same positions, fixed with 10% formalin, embedded and sectioned in paraffin and stained with HE. Pathological changes were examined under a light-microscope and the toxic effects were quantified.

1.10 Measurement of carboxyhaemoglobin (COHb) in blood

Carboxyhaemoglobin (COHb) is a stable complex of carbon monoxide and hemoglobin that forms in red blood cells when carbon monoxide is inhaled. Tobacco smoking through carbon monoxide inhalation raises the blood levels of COHb [30,31]. The COHb levels in the blood were measured at 450 nm with a kit (Shanghai Yope Biotechnology Co. LTD).

1.11 Statistical analysis

ANOVA was used to estimate overall significance followed by post hoc Tukey's tests corrected for multiple comparisons [32]. Data was presented as mean±SEM. A probability level of 5% (P<0.05) was considered to be significant.

2 Results

2.1 Human test

A human test for the cessation effect of a tea filter on smoking was performed in the Addiction Branch, Beijing Military Region General Hospital. In one trial, healthy male cigarette smokers who consumed approximately 14 ciga-

rettes per day on average were recruited and randomly divided into 2 groups (double-blinded, placebo-controlled): smoking with tea filters or with regular filters. After using the tea filter for 1 month, the average daily cigarette consumption decreased about 43% in the tea filter group. By contrast, no change in average daily cigarette consumption was detected in the control group using regular filters (Figure 1A). As a consequence of the reduction of cigarette consumption, the levels of exhaled carbon monoxide and urine cotinine content in the volunteers who smoked with tea filters were respectively significantly decreased by about 52.6% and 26.3% (Figure 1B). The test was discontinued for the control group, and the tea filter group was followed for an additional month. After using the tea filter for 2 months, the average daily cigarette consumption was decreased by about 56.5% (31.7% of the smokers quit smoking, 13.9% of the smokers reduced their cigarette consumption from 14 d^{-1} to 1-5 d^{-1}; 8.9% and 31.7% of the smokers respectively reduced their daily cigarette consumption by 60% and 30% and 13.9% of them did not change their cigarette consumption) (Figure 2A).

In another trial, we tested the effect of the tea filter on heavier smokers who had a stronger desire to quit smoking. 59 healthy male cigarette smokers were recruited and tested to smoke with tea filters for 3 months. The result showed that their average daily cigarette consumption respectively decreased by about 48%, 83% and 91% after using the tea filter for 1, 2 and 3 months and the average daily cigarettes consumed decreased to about 3 d^{-1} in the last month (Figure 2B), (the levels of exhaled carbon monoxide and urine cotinine content in the volunteers were significantly decreased), suggesting that the tea filter is effective for smoking cessation. The efficacy of the tea filter on smoking cessation is better than many other methods reported [7–14]. In addition, most subjects described that sputum and their smoking-related symptoms were reduced compared to the control group. Physical examinations of the subjects did not reveal any apparent side-effects.

2.2 Effect of theanine on the rewarding effect induced by nicotine

The conditioned place preference (CPP) paradigm is a measure of nicotine reinforcement. To find which materials in the tea filter are responsible for smoking cessation and to elucidate the smoking cessation mechanisms of the tea filter, we examined the effect of various components in the tea filter on nicotine induced reinforcement using the CPP method in a mouse model. Nicotine induced reinforcement was induced by daily injection of the mice with nicotine (0.5 mg kg^{-1} d^{-1}) for 7 d, while different compounds isolated from the tea filter were administrated 15 min before each nicotine injection. The results revealed that theanine (500 mg kg^{-1}), an amino acid derivative component of tea, had a similar effect in mice to DHβE, an inhibitor of nAChR, but green tea polyphenols (250 mg kg^{-1}) and caffeine (2 mg kg^{-1}) had no effect on nicotine induced reinforcement in the animals (Figure 3). The inhibition effects of theanine appeared to be time and dose dependent. While administration of theanine (250 mg kg^{-1} and 500 mg kg^{-1}) for 7 d respectively inhibited nicotine induced reinforcement about 25% and 50%, the inhibition of nicotine induced reinforcement was respectively about 90% and 95% after 2 weeks of theanine treatment for both doses.

2.3 Effects of theanine on the expression of the nicotine receptor (nAChR) in mouse brains

It has been shown that nicotine treatment increases the expression of nAChR while inhibition of nAChR and its related processes causes smoking cessation [33–36]. To study the cessation mechanisms of theanine on nicotine dependence, we next investigated whether or not theanine caused nicotine cessation by affecting nicotine-induced expression of nAChR using a mouse model. After daily injection with nicotine for 2 weeks, the protein levels of nAChR subunits

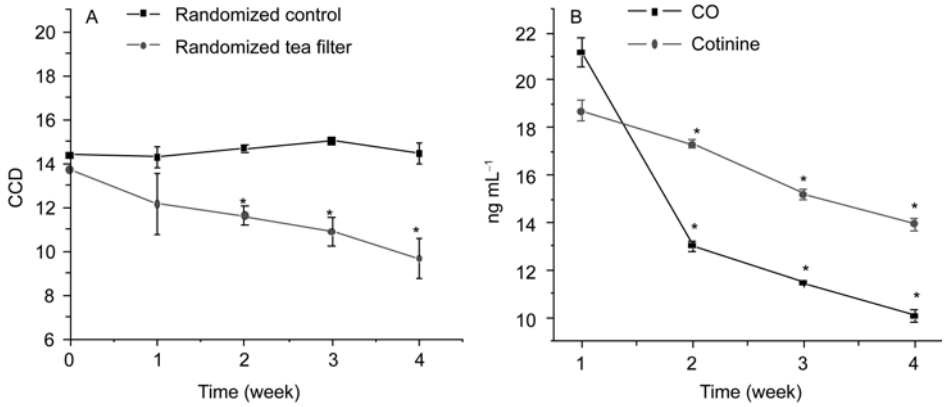

Figure 1 Smoking using tea filters significantly reduced the number of cigarettes consumed by volunteer smokers for one month. A, Average number of cigarettes consumed daily (CCD) by each volunteer smoker before (week 0) and after (week 1–4) using a regular or a tea filter; B, Effect of a tea filter on exhaled CO and urine cotinine excretion of volunteer smokers; *, $P<0.05$ when compared to week 0. Details are described in "Methods".

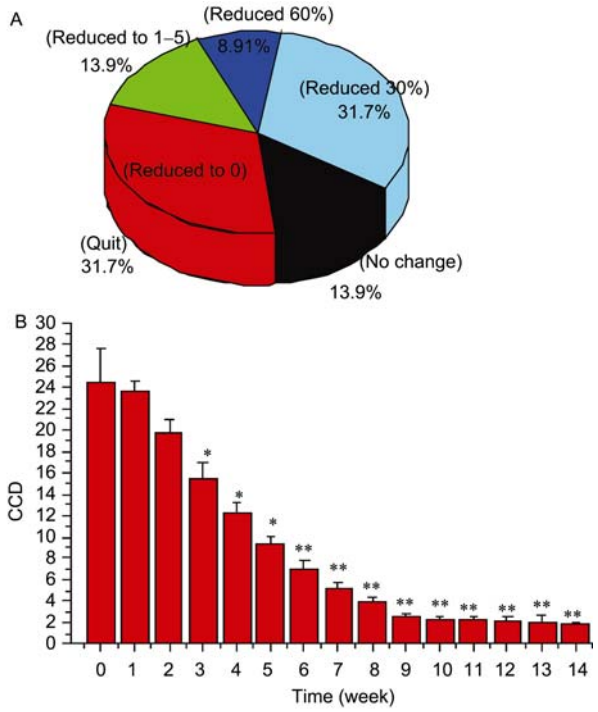

Figure 2 Smoking using tea filters significantly reduced the number of cigarettes consumed by volunteer smokers for 2 and 3 months. A, The percentage of the smoker population and their average daily cigarette consumption after using a tea filter for 2 months; B, the average number of cigarettes consumed daily (CCD) by each volunteer smoker before (week 0) and after (week 1–14) using a tea filter. *, P<0.05 when compared to week 0; **, P<0.01 when compared to week 0. Details are described in "Methods".

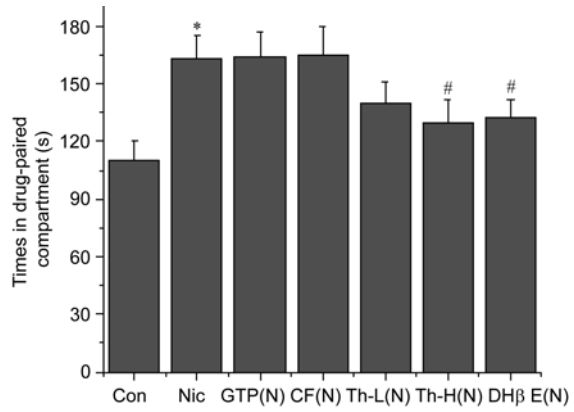

Figure 3 Effects of different compounds on nicotine dependence. The mice were treated with nicotine or different compounds in physiological saline every day for 9 d. The nicotine dependence of the mice was examined on the 10th day by a conditioned place preference (CPP) test. Con, mice were treated with physiological saline; Nic, mice were treated with nicotine (0.5 mg kg^{-1} d^{-1}); GTP(N), mice were treated with nicotine (0.5 mg kg^{-1} d^{-1}) and green tea polyphenols (250 mg kg^{-1}); CF(N), mice were treated with nicotine (0.5 kg^{-1} d^{-1}) and caffeine (2 mg kg^{-1}); TH-L(N), mice were treated with nicotine (0.5 mg kg^{-1} d^{-1}) and theanine (250 mg kg^{-1}); TH-H(N), mice were treated with nicotine (0.5 mg kg^{-1} d^{-1}) and theanine (500 mg kg^{-1}); DHβE(N), mice were treated with nicotine (0.5 mg kg^{-1} d^{-1}) and DhβE (2.0 mg kg^{-1}). The results are presented as mean±SEM, n=8. *, P<0.05, compared with control; #, P<0.05 compared with the nicotine group. Details are described in "Methods".

α4, α7 and β2 were increased in mouse brains. Theanine pretreatment significantly inhibited the induction of nAChR subunits in the brain (Figure 4). Therefore, the cessation effect of theanine on nicotine dependence may be attributed to its inhibition on nicotine-induced expression of nAChR subunits.

2.4 Effects of theanine on dopamine release in mouse brains

The increase of dopamine (DA) release is a significant reward process caused by nicotine [37]. We examined whether or not theanine had any effect on the DA release induced by nicotine in mice. After nicotine injection, the levels of DA were significantly increased in the striatum of the mouse brains. Pre-treating animals with theanine before nicotine injection significantly reduced the elevation of DA (Figure 5) induced by nicotine.

2.5 Detoxification effect of a tea filter on acute toxicity caused by cigarette smoking

Previous studies showed that the tea components, green tea

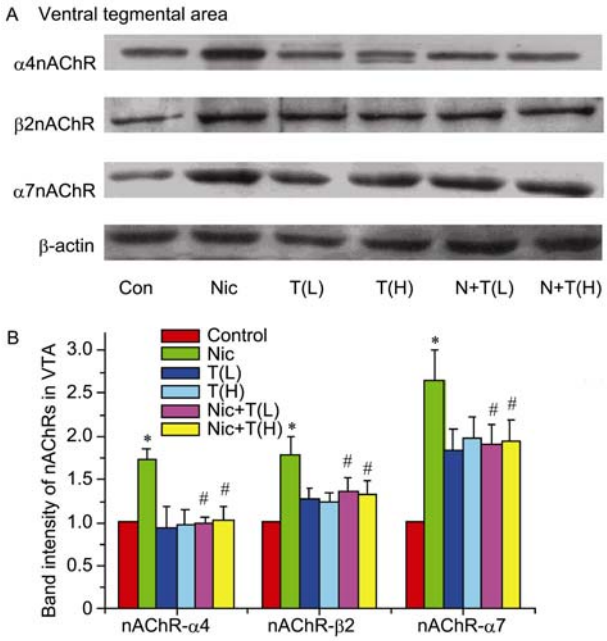

Figure 4 Effects of theanine on the expression of the nicotine receptor (nAChR) in mouse brain. Mice were daily treated with nicotine (0.5 mg kg^{-1} d^{-1}) for 2 weeks with or without theanine administered 15 min before nicotine injection. Protein extracts prepared from mouse brains were analyzed using Western blotting (A, B). The expression of nAChR was examined by Western blotting. Details of the procedures are described in "Methods". Control, mice were treated with physiological saline; Nic, mice were treated with nicotine; T(L), mice were treated with theanine (250 mg kg^{-1}); T(H), mice were treated with theanine (500 mg kg^{-1}); Nic+T(L), mice were treated with nicotine and theanine (250 mg kg^{-1}); Nic+T(H), mice were treated with nicotine and theanine (500 mg kg^{-1}). Data was analyzed by the ratio of band intensity of nAChRs over that of -actin, expressed as ratio±SEM, n=4. *, P<0.05, compared with the control; #, P< 0.05 compared with the nicotine group.

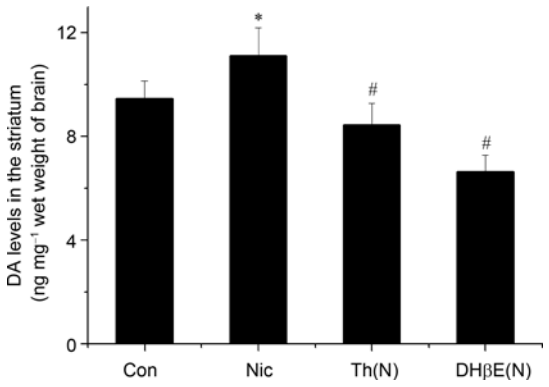

Figure 5 Effects of theanine (0.5 mg kg^{-1} d^{-1}) on nicotine-induced dopamine release. The mice were injected with nicotine (0.5 mg kg^{-1} d^{-1}) or physiological saline every day for 9 d. Different concentrations of theanine were injected alone or 15 min before nicotine injection. The levels of dopamine in the mouse brain were measured by HPLC with electrochemical detection**.** The details of the procedure are described in "Methods". Con, mice were treated with physiological saline; Nic, mice were treated with nicotine; Th(N), mice were treated with nicotine and theanine; DHβE(N), mice were treated with nicotine and an inhibitor of nAChR, DHβE. The results are presented as the mean±SEM, n=3. *, P<0.05, compared with the control; #, P<0.05 compared with the nicotine group.

polyphenols, and a tea filter scavenged the tar, free radicals, nitrosamine, [a] pyrene, benzo [a] anthracene, chrysene and total polycyclic aromatic hydrocarbons (PAHs) generated in cigarette smoking [18–23]. We further studied the efficacy of tea filters on the acute toxicity of cigarette smoke and found that the survival time of animals respectively increased by 32.2% and 60% by complex (half tea filter/half cellules acetate) and full tea filters and the acute toxicity of cigarette smoke was significantly reduced (Table 1). In the control group (cigarette with normal filters) marked congestion and haemorrhage in lung tissue was observed in 80% of mice. The tea filters reduced the number of mice with these pathological changes to 40% (results not shown).

2.6 Detoxification effect of a tea filter on mutagenicity caused by cigarette smoking

We investigated the effect of a tea filter on the incidence of micronuclei in polychromatic erythrocytes (PCE) as a measure of the mutagenicity ratio and as an indicator of toxicity in rats exposed to cigarette smoke for 75 d. The incidence of micronuclei in PCE significantly increased when rats were chronically exposed to cigarette smoke, in accordance with a mutagenic activity of cigarette smoke. When a cigarette with a tea filter was used, the incidence of micronuclei in PCE was inhibited by about 46% as compared to the rats which inhaled smoke from cigarettes with normal filters (Table 2). Hence there was a significant reduction in the mutagenicity of cigarette smoke by tea filters.

2.7 Effects of a tea filter on carboxyhaemoglobin (COHb) in mouse blood

Carboxyhaemoglobin (COHb) is a stable complex of carbon monoxide and hemoglobin that forms in red blood cells when carbon monoxide is inhaled. Tobacco smoking through carbon monoxide inhalation raises the blood levels of COHb [30,31] causing cardiovascular and cerebrovascular damage and diseases, e.g. neurasthenia, myocardium damage and atherosclerosis. In order to study the effect of a tea filter on the toxicity of CO generated from cigarette smoking, we measured the COHb levels in the blood. It was found that the COHb levels in the blood of the mice exposed to smoke from cigarettes with a normal filter were increased about 561% compared with the mice without such exposure, while the COHb levels in the blood of the mice exposed to smoke from cigarettes with a tea filter were decreased about 53% compared with the mice exposed to smoke from cigarettes with normal filters (Table 2). These

Table 1 Inhibition effect of a tea filter on the acute toxicity of cigarette smoke for mice

Groups	n	survival time (min)	effect
Smoking (normal filter)	10	11.25±0.56	
Smoking (complex tea filter)	10	14.85±0.77*	+32.2(%)
Smoking (whole tea filter)	10	18.00±0.68*	+60(%)

*, P<0.05 compared with smoking with a normal filter.

Table 2 Protective effect of a tea filter against mutation and COHb increase in blood caused by cigarette smoking

Groups	n	PCE/NCE	effect	HbCO (mg/mL)	effect
Control (no smoking)	5	1.85±1.42	1.27±0.66		
Smoking (normal filter)	5	14.55±7.06#	+687%	8.40±0.42#	+561%
Smoking (tea filter)	5	5.68±2.10*	–60%	3.98±0.99*	–53%

#, P<0.05 compared with a control (no smoking); *, P<0.05 compared with smoking with a normal filter.

results suggest that a tea filter inhibited the COHb levels generated in cigarette smoking and prevented cardiovascular and cerebrovascular diseases caused by cigarette smoking.

2.8 The detoxification effect of a tea filter on lung damage caused by cigarette smoking

Specimens of lungs from each animal from all groups were examined under the optical microscope for pathological changes. A variety of pathological alterations were discovered in the lungs of the control group. Tea filters significantly reduced these pathological changes. An increased broken alveol, thickness of bronchi capillary walls with signs of neutrophil and lymphocyte infiltration was observed. There was an increase of inflammatory exudate and lympho-proliferation in the bronchial lumen (Figures 6B and C). Abscess and low fiber proliferation were found in the lung tissue in 4 rats and 1 rats showed minor pathological changes from the normal filter group. Only 1 rat showed the pathological alterations and 1 rat showed minor pathological changes in the lungs (moderate neutrophil and lymphocyte infiltration of alveoli) (Figure 6D), and in 3 animals almost normal lung tissue was observed from the tea filter group.

3 Discussion

Cigarette smoking addiction is caused by the interaction of nicotine with the nAChR in the brain [33–36]. Our animal experiments showed that theanine in the filter exerted an inhibition effect similar to the nicotine acetylcholine receptor (nAChR) inhibitor. To further improve the mechanism of the inhibition effect of theanine on nicotine dependence, we studied the effect of theanine on the expression of nAChR. The results showed that theanine significantly inhibited the nicotine-induced expression of nAChR (Figure 4). The increase of dopamine (DA) release is a fundamental reward process caused by nicotine [37]. Our results showed that pre-treating animals with theanine before nicotine injection significantly reduced the elevation of the DA level in mouse brains.

To determine whether or not theanine in tea filters is readily inhaled into the lungs, the hot gas steam from a burning cigarette with a tea filter was collected using a specially designed smoking instrument and the amount of theanine was measured. It was found that approximately 65 μg of theanine was brought out and entered into the hot gas stream after smoking one cigarette with the filter, about 10 μg mL^{-1} in the plasma of animals after inhaling the smoke from 7 cigarettes with tea filters, suggesting that theanine is readily inhaled into the lungs and probably enters the pulmonary circulation of the smoker. Once in the blood, theanine passes through the blood-brain barrier and reaches targets in the brain [38], preventing the development of nicotine dependence. Because there are more than 450 kinds of compounds in tea, it is possible that components other than theanine in the tea filter also have a tobacco cessation effect or they may act synergistically with theanine to promote tobacco cessation. Further studies on other tea components may reveal additional tea components which are factors in the tobacco cessation effect.

The animal experiments also showed that the tea filter significantly reduced the acute and chronic toxicities as shown in tables 1, 2 and Figure 6. Our previous work has shown that tea components, the green tea polyphenols, scavenge the tar, free radicals, nitrosamine, [a] pyrene, benzo [a] anthracene, chrysene and total PAHs and protect cells from cigarette smoke-induced toxicity [8–23], so the detoxification effects of tea filters may mainly come from green tea polyphenols. These results indicated that tea filters not only helped smokers to quit smoking but also reduced the toxicity induced by cigarette smoking.

Cigarette smoking is the major risk factor for a series of life threatening diseases including cancer and heart attack, which causes millions of deaths each year worldwide [1–5]. Different cigarette filters have been developed with the purpose of reducing such harmful chemicals as tar and nicotine in tobacco smoke. However, a smoker may smoke more cigarettes using these filters, inhale more deeply or decrease the time between puffs to compensate for the desired nicotine intake, leading to exposure to equal or greater doses of the toxic and cancer-causing substances present in cigarette smoke [39]. Therefore, smoking using these filters is not an alternative for lowering the risk of smoking-related diseases.

Smoking cessation is the ultimate method for reducing smoking-related diseases. However, quitting smoking is extremely difficult due to the addictive nature of nicotine. Such smoking cessation methods as nicotine replacement therapy (NRT) and nAChR partial agonists and antagonists have been shown to help some smokers quit, but they are also reported to have high relapse rates [7–14]. In addition, some of these methods are perceived as being inconvenient. For example, nicotine products such as nicotine spray and gum have to be frequently replaced, while nAChR partial agonists are prescribed medications and are administrated as drugs. These methods are not easily psychologically accepted by the smokers who want to quit, affecting the efficacy of their smoking cessation.

The novel tea filter treatment might avoid the pitfalls mentioned above and effectively promote smoking abstinence. Because it uses the smoking process to help quit smoking**,** it may be accepted by smokers with less psychological obstacles and side effects. When a smoker is smoking using the tea filter, the inhibitors of the nicotine receptor in the tea filter are absorbed through the respiratory system and travel to the brain where they exert cessation effects. This appears as a circulatory process moving towards smoking cessation, which continues until the smoker quits smoking (Figure 7).

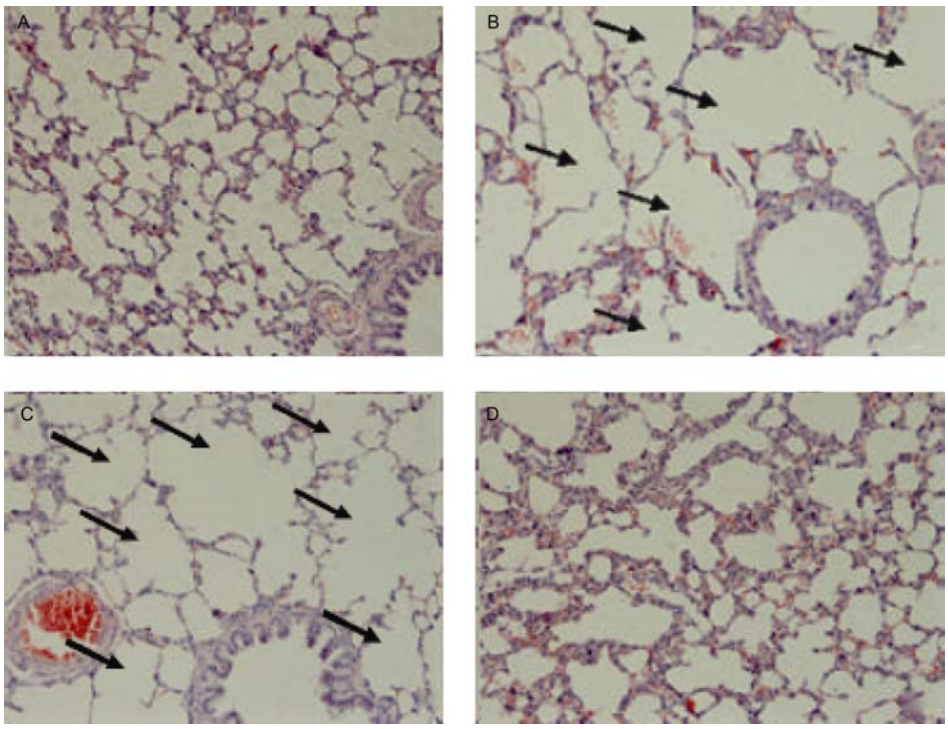

Figure 6 Effect of a tea filter on the histopathology of the lung tissue of rats treated with cigarette smoke. A, Normal animals without being treated with cigarette smoke; B and C, animals treated with normal cigarette smoke and D, animals treated with smoke generated from cigarettes with tea filters for 75 d. A variety of pathological alterations were discovered in the lungs. Most significantly, many of the alveoli were found broken when animals were treated with normal cigarette smoke (arrows in B and C), while using a tea filter almost reversed this effect. Specimens were taken from the same position of the lung tissue of the rats, fixed with 10% formalin, embedded in paraffin, sectioned and stained with HE. Pathological changes were examined under a light-microscope. Details are described in "Methods".

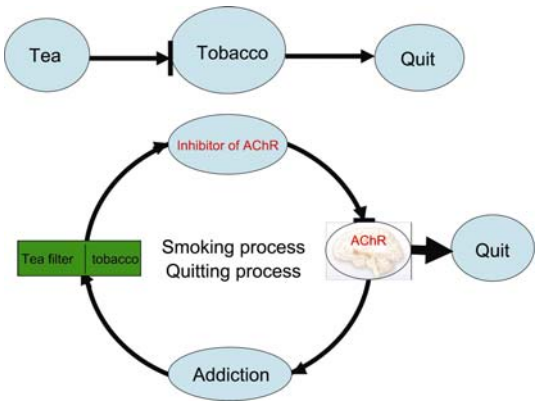

Figure 7 Tea filter inhibits cigarette smoking addiction through a circulative process moving towards smoking cessation.

Smoking is one of the largest international public health problems. For example, in China, the population of smokers is about 350 million and is increasing. Similar situations are found in India and other developing countries [3,40]. The tea filter is produced by a slight and inexpensive modification of the existing filter which is suitable for developing countries.

In conclusion, this work proposed a tea filter and theanine, for inhibiting smoking addiction through inhibition of nAChR and provides an effective method for treating tobacco addiction.

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