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Original Article

Efficacy of Combined Relaxed Deep-Breathing with Chest Mobilization Exercise and *Vernonia cinerea*-Hard Candy on Smoking Cessation and Oxidative Stress in Active Teenage Smokers

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ABSTRACT

Background: Smoking cessation is very important worldwide. Chronic smoking can induce oxidative stress and inflammatory status and induce dangerous diseases such as hypertension and lung cancer. Standardized counseling is an important process in a routine program for smoking cessation. Withdrawal symptoms from smoking cessation are a significant barrier to a successful result, and they can be relieved by relaxed deep-breathing exercise. At present, the Thai herb, *Vernonia cinerea* (VC), has been claimed to reduce cigarette smoking because of its antioxidant compounds and nicotine that are modified and used as lozenges, gum, and hard candy. However, its efficacy in smoking cessation has not been confirmed. Furthermore, the efficacy of relaxed deep-breathing (rDB) during the chest mobilization exercise (CME) with VC hard candy on smoking cessation and oxidative stress is unclear. **Objective:** This study aimed to evaluate the combined effects of rDB/CME and VC-hard candy on smoking cessation and oxidative stress status in active teenage smokers. **Methods:** Hard candy with honey and VC powder from whole mixed parts of the stem, flowers and leaves was developed industrially under the spray dry technique. Thirty active smokers were randomized into three groups; product group (rDB/CME+ product) (aged 25.0 ± 3.0 years, n = 10), placebo group (rDB/CME + placebo) (aged 26.9 ± 3.7 years, n = 10), and a control group with no product or placebo administered (aged 25.6 ± 2.7 years, n=10). All of the groups received consultation on specific smoking cessation and two weeks of strict observation, which was followed up for 8 weeks. The 7-day point prevalence abstinence rates (7-day PAR) and continuous abstinence rate (CAR) were reported at week 2, 4, 6 and 8. In addition, the oxidative stress status with lipid peroxide and glutathione (GSH) in blood was evaluated before the program and after 2 weeks. **Results:** The results of 7-day PARs in the control group showed no statistical changes at week 2 (0%), 4 (10%), 6 (20%) and 8 (20%), which was the same in the rDB/CME + placebo group (10%, 20%, 30% and 40%, respectively). Whereas, a significant difference was presented in the rDB/CME+ product group (20%, 60%, 80% and 90% respectively). When comparing between the groups, 7-day PARs at week 2 was not statistically different, but it was in the follow-up period at week 4, 6 and 8. There was no statistical difference at week 4 between the three groups, but there was between the rDB/CME+ product, control and rDB/CME+ placebo groups at week 6 and 8. The results of CAR showed no statistical difference between the control and rDB/CWE+ placebo group in any of the periods. Whereas the rDB/CWE+ product group showed a significant difference after week 4. The CAR was statistically different between the groups after week 6 and 8. At week 6, the CAR of the rDB/CWE+ product group was different to the control group. There was no difference between the control and rDB/CWE+ placebo groups, or between the rDB/CWE+ product and placebo groups. At week 8, the CAR of the rDB/CWE+ product group was different from that of the control, but not from the rDB/CWE+ placebo group. Finally, the GSH level increased significantly in the rDB/CWE + product group when compared to the rDB/CWE+placebo group. Moreover, malondialdehyde (MDA) levels decreased significantly in both the placebo and product groups. In addition, MDA levels showed a significant difference between baseline and after 2 weeks in the rDB/CWE + placebo and product groups. **Conclusion:** Integrating relaxed-deep breathing with chest mobilization exercise and VC hard candy for 2 weeks can help smoking cessation during consultation, and possibly reduce oxidative stress status among active teenage smokers.

Key words: 7-day point prevalence abstinence rate, Chest mobilization exercise, Continuous abstinence rate, Oxidative stress, Relaxation deep-breathing, Smoking cessation, *Vernonia cinerea*-hard candy.

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INTRODUCTION

Smoking prevalence in Thailand has been higher among people with low levels of education and income. Data in 2019 indicated that the number of smokers might increase globally to 1.6 billion over the next 25 years,[1] but no updated data have reported a changing trend after the COVID-19 pandemic. Although the overall number of cigarette smokers decreased from 12.2 million to 10.86 million between 1991 and 2007, the number of younger men (aged around 18 years) and women (aged about 22 years) smokers increased.[2] The Framework Convention of Tobacco Control (FCTC) from the World Health Organization (WHO) was the first international health treaty to be endorsed by 180 countries, including Thailand, and it reported increased annual consumption of over 500 cigarettes per adult.[3] Therefore, The Thai Health Professional Alliance against Tobacco (ThaiPAT) was established in late 2005 and has been campaigning since 2007.[4] The Thai government has been campaigning for a reduction in the smoking rate by providing various services such as behavioral counseling and/or suggestions on how to quit

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smoking by using the one-stop service or 1,600 telephone lines, as well as smoking cessation clinics in many hospitals. Previous evidence has shown that behavioral counseling with pharmacotherapy *via* nicotine replacement therapy (NRT) is successful.[5,6] Present data on smoking cessation can be practiced pharmacologically with nicotine gum, transdermal nicotine or varenicline or bupropion drugs and a non-pharmacological program.[7] The basic smoking cessation program comprises "5A": ask, advise, assess, assist and arrange a follow up by the health team.[8,9] Although using NRT increased the rate of smoking cessation by 50-70%,[10] there is not only the major disadvantage of high-cost nicotine replacement,[11] but also various adverse effects such as skin irritations, soreness of the mouth and throat, mouth ulcers, hiccups, and coughing[12] as well as withdrawal symptoms, which are the main factor of success or failure in attempts to quit smoking.[13] Previous data reviewed that application of alternative therapy such as yoga, meditation and relaxed breathing can relieve both stress and withdrawal symptoms.[14] Evidence supported by 30-minute deep breathing[13] or relaxation with a deep breathing exercise[15] can be applied with the chest mobilization technique.[16] Therefore, relaxation with deep breathing exercise can be used during smoking cessation,[17] but with only a 37% success rate.[15] Therefore, smoking cessation with a pharmacological agent also is important. A search for an anti-smoking agent from natural plants instead of drugs has been proposed in Thailand. For example, medicinal herbal tea from China, containing *Eugenia aromaticum* and *Astragalus membranaceus* Bunge, underwent a 4-week trial to reduce smoking withdrawal symptoms, and it was successful in 38% of active smokers who had stopped smoking.[18] Furthermore, *Vernonia cinerea* (VC) was reported in Thai traditional medicine as an anti-smoking aid, and has been studied in a special stop-smoking clinic at Thanyarak Institute in Pathumthani. The results of a 14-day VC tea supplementation program showed a higher abstinence rate (28.1%) when compared to the control group (21.9%).[19] Moreover, the efficacy of VC juice on smoking rate was studied preliminarily among light smokers and the results showed that the smoking rate for light cigarettes decreased by approximately 63% when compared to the control group (14 %).[20] Previous evidence found that some active compounds, such as flavones and flavonol in methanolic VC extract, possess a strong inhibitory effect on the human cytochrome, P450 2Ag (CYP2A6), and monoamine oxidase (MAO-A and MAO-B), which function on catalyzing nicotine and dopamine metabolisms, and could have implications on combining drug therapy with smoking cessation.[21] Whereas, crude VC leaf extract boiled in water showed flavonols, flavones, nitrate, nitrite, and nicotine.[22,23] Therefore, VC can be used for reducing cigarette smoking. Various VC packages were developed in Thailand from teabags to new products such as lozenges, gum or candy. Furthermore, the process of preserving active compounds in VC extract can be performed with dry extract before mixing in lozenges, gum or candy. In a previous review, the most frequent drying methods such as freeze, spray, spray freeze and supercritical fluid drying, improved the stability and bioavailability of dry materials.[24] Drying can be performed using evaporation mechanisms, such as vacuum or foam drying; evaporation and atomization pathways like spray drying (SD); sublimation mechanisms like freeze-drying (FD); and spray freeze and supercritical fluid drying.[25] When comparing between FD and SD, FD is suitable for processing temperature sensitivity, low temperature, higher yield, and greater production, but SD is simple, convenient and cost-effective with a short processing time. Whereas, spray freezing and supercritical fluid drying come at a very high cost.[26] Therefore, SD was suitable for VC extract preparation before manufacturing the product. Updated data presented total flavonoids and nicotine that were slightly higher in SD extract, which was the same as the activity on inhibitory 2,2-Diphenyl-1-picrylhydrazyl (DPPH) compared to FD extract.[27] Finally, SD extract was newly developed industrially for VC-hard candy by mixing with glucose syrup and refined glucose and sealed in a blister pack. Therefore, this study aimed to evaluate the efficacy of combined relaxed deep breathing with chest mobilization and VC-hard candy on smoking cessation among active teenage smokers in Chiang Mai province.

MATERIALS AND METHODS

Product preparation

Industrial VC product preparation followed that in a previous study.[27] Before starting this study on active smokers, raw materials of VC were purchased from an organic farm in Phitsanulok province, Thailand. Concerning safety guarantees in raw materials, mercury and zinc were analyzed by the In-house method, TE-CH-260, in connection with AOAC (2016) 2013.06 and AOAC (2016) 999.10, in the same way that tin is analyzed by the In-house method TE-CH-340 based on AOAC (2016) 985.16 at the Central Laboratory (Thailand) Co., Ltd. (Chiang Mai, Thailand). Twenty grams of whole plant parts; the stem, flowers and leaves were dried in a heated oven and mixed with 350 mL of sterile water before boiling at 60 degrees Celsius. Finally, approximately 150 mL of condensed VC juice was filtered before the dry extract was prepared by SD at the Argo-industrial Business Service Center, Faculty of Agro-industry, Chiang Mai University, Thailand.

The VC-product was manufactured industrially in accordance with a previous study.[27] The higher concentration of nicotine in the extract was selected for making the pilot anti-smoking product, designed as hard candy. Standardized manufacturing of the hard candy was performed under Certificate TH 14/7924 (TAS 9023-2007) GMP Codex Alimentarius, the Recommended International Code of Practices, and General Principles of Food Hygiene, CAC/RCP 1-1969, Rev.4 (2003) at the Chiang Mai Healthy Product Co., Ltd. (Chiang Mai, Thailand). The hard candy was contained in a sealed candy panel. Each piece contained VC extract, glucose syrup (Capital Glucose Co., Ltd., Nakornpathom, Thailand), and refined sugar (Mitr Phol Sugar Corp., Ltd., Lampang, Thailand), whereas the placebo candy had only glucose syrup and refined sugar. The production process involved dissolving ingredients and cooking before dehydrating and cooling, and kneading and molding the semi-product before sorting it into hard candy. Finally, eight pieces of candy were packed into a sealed blister pack and used in this study within 6 months (Figure 1). All of the processes were performed under sterile techniques with industrial pharmacists.

Sample size calculation

Statistical calculation of sample size was performed by following a previous study[19] between groups of controls (n=34) and crude VC supplement in teabags (n=32). The main outcome of the 7-day point prevalence abstinence rates (7-day PARs) of 37.5% in the VC

Figure 1: Hard candy containing VC extract from SD mixed honey produced by the Chiang Mai Healthy Product Co., Ltd. (Chiang Mai, Thailand).

supplement, and 34.4 % in the control group, were calculated under a G*Power (version 2.1.9.4) (Franz Faul, University Kiel, Germany), with 1.34 of effect size (*dz*), 0.05 of alpha error prob and 0.80 of Power (1- beta err prob). Eleven subjects were suggested for each group. The inclusion criteria of participants in this study were being an active smoker, aged 18 years, been smoking for one year, and having at least three scores of nicotine dependency from the Fagerstrom test for nicotine dependence.[28] Subjects being treated with other anti-smoking medicine, pregnant, unwilling to stop smoking, ill with diseases such as renal, heart or pulmonary conditions, or having abnormal completed blood count (CBC), renal and liver results, and those who could not communicate in the Thai language and did not want to join the study were excluded. Thirty active smokers were randomized into three block stratified groups of control, placebo and product. All of the groups received the basic smoking cessation consulting program following the ENP Guidelines for Testing Tobacco Dependence (2016),[29] whereas the placebo and VC product groups were taken and trained for relaxed deep breathing with chest mobilization exercise (rDB/CME) by an expert physical therapist.

The study program was approved by the Ethical Human Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (Study code AMSEC-62FB-004), and all of the participants signed a consent form before starting the program. Before and after 2 weeks of study, vital signs, CBC, and renal and liver function were re-evaluated for any adverse effects. Moreover, the 7-day PARs and continuous abstinence rate (CAR) at week 2, 4, 6 and 8 were reported. Failure to use the product or placebo for more than 2 days per week and use of extra-antioxidant supplements during the study period were not allowed.

The relaxed deep breathing with chest mobilization exercise (rDB/CME) program

This program was designed by combining relaxed deep breathing and chest mobilization exercises. The relaxed deep breathing (rDB) exercise was proposed and approved for reducing withdrawal symptoms and cigarette cravings.[13] The basic guideline for deep breathing was slow inspiration for 5-seconds through the nose, holding breath for 2 seconds, before slow expiration for 5-seconds through the mouth. Five repeated cycles of deep-breathing per time and ten times with 30-second rest intervals in the morning, mid-day, evening, and before sleeping periods were recommended.[13,30,31] In this study, rDB/CME was designed with four patterns[31] (Figure 2).

Relaxed deep breathing with lower costal expansion exercise. Back of the hands are placed beside the lower coastal areas, with slow deep inspiration, and then breath held for 3 seconds before slow expiration (Figure 2A).

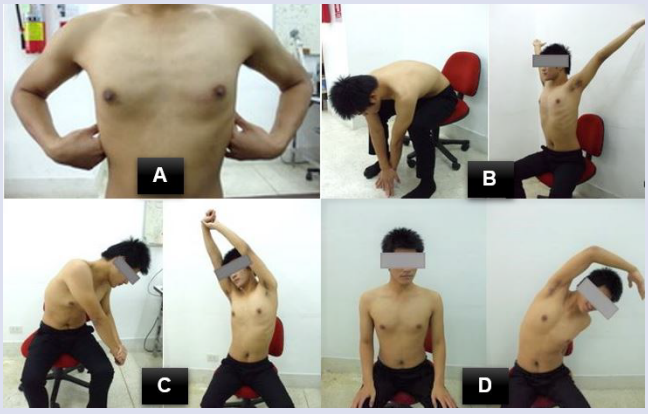

Figure 2: Chest mobilization techniques; lower costal deep breathing exercise (A), V-pattern (B), trunk rotation (C), and lateral flexion (D).

V-pattern chest mobilization (CM): the participant sits on a chair with feet on the floor. The trunk is fully flexed forward from the hips with hands touching the floor. Then, expiration before lifting the body with bilateral elevation and slight abduction of both arms over the head with full inspiration (Figure 2B).

Trunk rotation CM: the participant sits on a chair with feet on the floor. Both arms are placed together by the left side of the trunk which is bent forward. Expiration starts before rotating the hands and arms up and over the head to the right side with full inspiration (Figure 2C). Then, the same is performed from right to left.

Lateral flexion CM: the participant sits on a chair with feet on the floor and a hand on each thigh. The right arm is lifted up over the head with full inspiration before returning to the starting position with expiration (Figure 2.D). The same is performed with the left arm.

Outcome evaluation

CBC, and renal and liver function were investigated in the laboratory by the Bangkok RIA Laboratory, Ltd., Chiang Mai province, Thailand.

7-day PARs at week 2, 4, 6, and 8 were calculated from non-smokers who had stopped smoking for at least 7 days before the evaluation date.[32]

The CAR at week 2, 4, 6, and 8 was calculated continuously from the number of non-smokers compared to all of the participants.[32]

Oxidative status parameters; glutathione (GSH) and malondialdehyde (MDA) were investigated in the laboratory. Three milliliters of whole blood were taken by a Medical Technologist and kept in anti-coagulant Ethylenediaminetetraacetic acid (EDTA). Whole blood at 400 µL was analyzed for GSH with a 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) assay,[33] whereas the plasma separated by centrifugation at 3,000 rpm for 10 min was analyzed by MDA using the Thiobarbituric acid reactive substances (TBARs) method.[34]

Statistical analysis

The normal distribution of all data was rechecked with the Kruskal-Wallis ANOVA test before being represented in the mean and standard error of measurement (SEM). The 7-day PARs and CAR between groups were analyzed statistically with the Chi-square test, whereas the GSH and MDA between groups and within a group were analyzed with the paired t-test. All statistical analyses were performed by the Statistical Package for Social Sciences (SPSS) software version. 10.0 (SPSS Inc, Chicago, IL, USA) for Windows at p less than 0.05.

RESULTS

From the small sample of participants, who were randomized into three groups, normal distribution was presented after the Kruskal-Wallis test was used statistically ($p > 0.05$). Therefore, the mean and standard deviation (SD) of all parameters were expressed. Characteristics of each group are presented in Table 1. The characteristics such as age, weight, height, body mass index, and smoking history such as packs/year or smoking period between the three groups were not statistically different ($p > 0.05$), except at the education level.

Efficacy of relaxed deep breathing with chest mobilization exercise on 7-day PARs and CAR

The 7-day PARs meant a non-smoking period of seven days before the assessment period at week 2, 4, 6 and 8. There was no statistical difference in the 7-day PARs during any period in the control group (0%, 10%, 20% and 20% respectively, $p = 0.67$), which was the same as

in the rDB/CME+placebo group (10%, 20%, 30% and 40% respectively, p = 0.62). Whereas, a statistical difference occurred in the rDB/CME + product group (20%, 60%, 80% and 90% respectively, p = 0.008). However, there was no statistical difference between the groups at week 2 (p = 0.79), but there was at week 4 (p = 0.02), 6 (p= 0.02) and 8 (p =0.009). At week 4, the 7-day PARs increased significantly in the rDB/CME+ product group when compared to the control group (p= 0.02), whereas no statistical difference occurred between the control and rDB/CME+ placebo group (p=0.54), which was the same between the rDB/CME+ placebo and product group (p = 0.07). There was a statistical difference between the rDVB/CWE + product group and control (p = 0.009) and rDB/CME + placebo group (p =0.009) at week 6. Finally, the 7-day PARs in the rDB/CME + product group showed a statistical difference from the control (p = 0.002) and placebo groups at week 8 (p =0.02). In addition, there was no difference between the control and rDB/CME + placebo group (p =0.34) (Figure 3A).

The results of CAR at week 4, 5 and 6 showed no statistical difference within the control group (10%, 20% and 20 %, p = 0.72), which was the same as in the rDB/CME + placebo group (20%, 40% and 40%, p = 0.11), but not the rDB/CME + product group (60%, 80% and 80%, p < 0.001). When analyzing between the groups in each period, there was no statistical difference at week 4 (p=0.08), but there was for week 6 (p = 0.04) and 8 (p = 0.04). At week 6, the CAR of the rDB/CWE + product group was statistically different to the control group (p = 0.02), but there was no difference between the control and rDB/CWE + placebo groups (p = 0.48) or between the rDB/CWE + product and placebo groups (p = 0.21). Finally, at week 8, the CAR of the rDB/CWE + product group was statistically different to that of the control group (p= 0.02), but no different from the rDB/CWE + placebo group (p = 0.21)

Effects of rDB/CWE with the product on oxidative stress status within 2 weeks

There was no statistical difference in GSH or MDA between the groups at the baseline period (p = 0.44 & 0.67), which was the same as at week 2 of the study (p = 0.81 and p = 0.48) (Table 2 & Figure 4). When comparing between baseline and after 2 weeks, the GSH levels in the control and rDB/CWE + placebo group were not statistically different (p = 1.00 & p = 0.95), except for the rDB/CWE + product group (p = 0.02). In addition, the MDA levels between baseline and after 2 weeks in the rDB/CWE + placebo and product groups showed a significant difference (p = 0.02 & 0.005). When analyzing the effect size (*dz*) at baseline and after 2 weeks, both GSH and MDA in the rDB/CWE + product group showed great effect size at 1.06 and 0.82, respectively, which was the same as in the rDW/CWE + placebo group (1.34).

DISCUSSION

This study represented the efficacy of a new anti-smoking product from a natural Thai plant, *Vernonia cinerea* (VC). A previous study on product preparation and safety investigation under the policy of the Ministry of Public Health (Thailand) for diet contaminant standards of heavy metals stated the safety criteria for study in humans.[27] Previous evidence showed different contents of total phenolic compounds, total flavonoids and nicotine in VC extracts from two preparative techniques; for example, mixing 20 g of raw dry VC materials in 3 cups of clean water before braising for a final 150 mL of condensed juice.[20] Catechins such as epicatechin gallate (ECG), epicatechin (EC), epigallocatechin gallate (EGCG), catechin, and flavonoids were found dominantly in freeze-dried VC leaf extract.[22] Whereas, total phenolic was presented in leaf extract when compared to the stem and flower. Moreover, VC showed antioxidant activity by scavenging the 2,2'-azino-bis (3-ethylbenz- thiazoline-6-sulphonic acid) (ABTS) cation and DPPH radicals.[22] The nicotine in VC extract from spray and freeze-dry techniques especially,[22] acted as a radical scavenger by binding with iron[35] and scavenging DPPH radicals as well as protecting erythrocytes from AAPH-and tert-Butyl hydroperoxide (BuOOH)-induced oxidative hemolysis.[36]

In a previous study, the efficacy of VC on smoking cessation within 2 weeks was studied.[20] The pilot hard candy produced by the Healthy Bee Co., Ltd showed 2.35±0.33 mg of nicotine per piece of candy. Previous evidence showed that nicotine use varied depending on the type of product; such as transdermal patches (5-52.5 mg),[37] nicotine gum (2 and 4 mg), nasal sprays (0.5 or 1.0 mg per spray), nicotine inhalator (10 and 15 mg), nicotine lozenges (1, 1.5, 2 and 4 mg strength) and nicotine sublingual tablets (3 mg).[38] Therefore, this study assumed that the nicotine and antioxidant activity of VC in the product might have the potential for smoking cessation. All of the participants were educated by following the basic smoking cessation program that comprised "5A": ask, advise, assess, assist, and arrange to follow up, respectively.[8,9] Previous evidence found that the consulting program by phone or individual face-to-face participation increased the smoking cessation rate by both physicians and non-physicians at 6 months (19.9

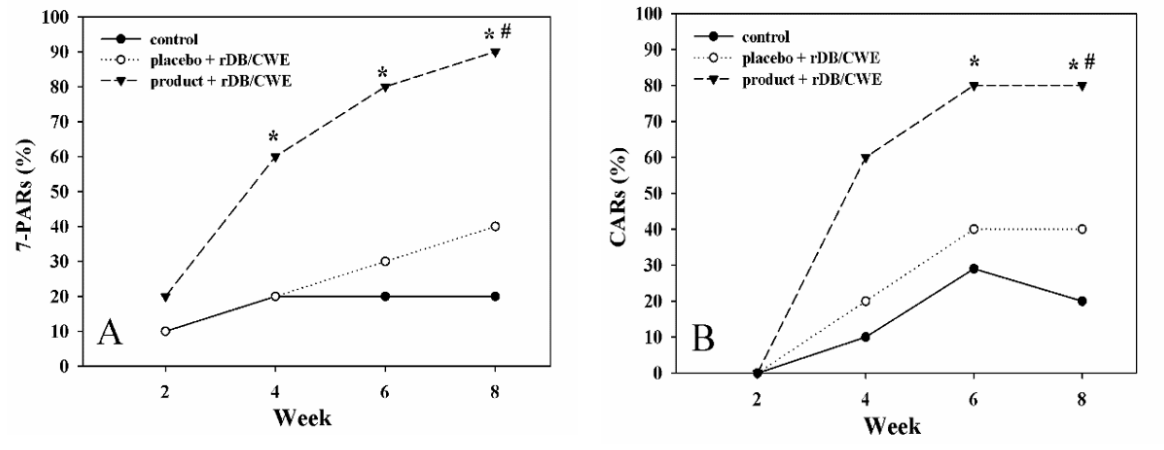

Figure 3: (A) The 7-day PARs and (B) CAR between the three groups; control, placebo and product, with relaxed deep breathing and chest mobilization exercise (placebo or product+ rDB/CWE) at week 2, 4, 6 and 8. * p< 0.05 when compared to another group in the same period and # p< 0.05 when compared to week 2, 4 and 6 within a group.

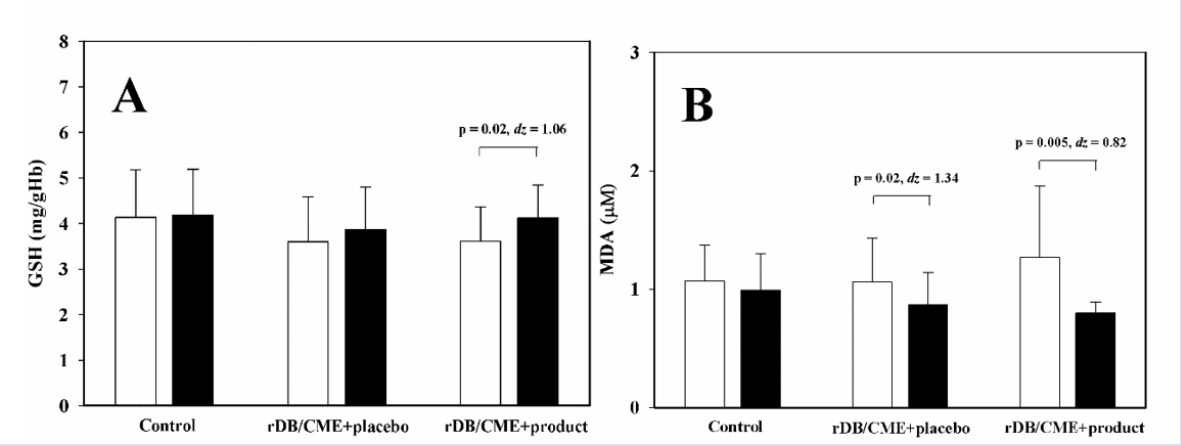

Figure 4: GSH (A) and MDA between the three groups before (white bar) and after 2 weeks (black bar). rDB/CME = relaxed deep breathing with chest mobilization exercise, *dz* = effect size.

Table 1: Characteristics of the participants in each group.

Parameters	Control	rDB/CME +Placebo	rDB/CME +Product	p
Characteristics				
Sex (male)	10	10	10	
Age (years)	25.60 ± 2.67 (18 - 42)	26.90 ± 3.71 (18 - 45)	25.00 ± 3.00 (18 - 41)	0.91
Weight (kg)	67.70 ± 2.73 (50 - 83)	62.40 ± 2.76 (48 - 82)	71.50 ± 4.54 (58 - 104)	0.19
Height (m)	1.67 ± 0.01 (1.56 - 1.77)	1.68 ± 0.02 (1.55 - 1.81)	1.69 ± 0.01 (1.62 - 1.74)	0.79
Body mass index (kg/m²)	24.02 ± 0.83 (18.37 - 27.53)	21.91 ± 0.86 (18.31 - 25.71)	24.80 ± 1.48 (19.16 - 34.75)	0.18
Packs/year	1.89 ± 0.74 (0.15 – 2.20)	2.48 ± 2.32 (0.30-8.0)	2.03 ± 1.17 (0.35 – 3.5)	0.27
Education - Vocational certificate - Bachelor degree	 6 4	 1 9	 10 0	0.03
Smoking period (years)	4.1 ± 5.67 (1 - 20)	6.1 ± 4.12 (2 - 16)	4.2 ± 2.25 (1 - 8)	0.05

Note: Data are presented in mean and standard deviation (SD) (min-max), rDB/CME = relaxed deep breathing during chest mobilization exercise, the p-value was analyzed from one-way ANOVA and the Chi-square test.

Table 2: Oxidative Stress Status of the three groups at baseline and after 2 weeks.

Parameters	Group								
	Control (n=10)		rDW/CWE+ placebo (n =10)		rDB/CWE+product (n=10)				
	Baseline	2 weeks	Baseline	2 weeks	Baseline	2 weeks	p*	p#	dz
GSH (mmol/gHb)	4.13 ± 1.05 (2.65 - 6.32)	4.18 ± 1.01 (2.61 – 5.63)	3.60 ± 0.99 (2.32 - 4.85)	3.87 ± 0.93 (2.43 - 4.87)	3.61 ± 0.75 (2.68 - 5.18)	4.12 ± 0.73** (2.73 - 5.18)	0.44	0.81	1.06
MDA (µmol/L)	1.07 ± 0.30 (0.77 – 1.70)	0.99 ± 0.31 (0.65 - 1.54)	1.06 ± 0.37 (0.68 - 1.91)	0.87 ± 0.27** (0.51 – 1.51)	1.27 ± 0.60 (0.84 - 2.75)	0.80 ± 0.09** (0.66 - 0.93)	0.67	0.48	1.34/0.82

Note: Data show the mean ± standard deviation (min-max). The p* value was compared between groups at baseline and p# value was compared between groups at week 2 by one-way ANOVA analysis, GSH = glutathione, MDA = malondialdehyde, *dz* = effect size from comparison between before and after 2 weeks within the group.

%, OR=2.2, 95% CI: 1.5-5.2 and 15.8%, OR= 1.7, 95% CI: 1.3-2.1), when compared to non-counseling (10%). In addition, 7-day PARs and CAR at week 8 were approximately 20%.[39]

Updated evidence on the efficacy of VC pastilles was reported in 2021. The result showed significantly higher CAR at week 12 when compared to the placebo group.[40] Whereas, this study showed high 7-day PARs and CAR at week 8 (40%) in the rDB with chest mobilization and placebo group. In addition, the result of the 7-day PARs and CAR was 90% and 80%, respectively, in rDB/CME with VC-product group, which was significantly higher than that in the rDE/CME with placebo group. The results in this study of 7-day PARs and CAR at week 2, 4, 6 and 8 between the control and placebo groups were not statistically different, except for the VC-product group, which seemed to have a higher percentage of CAR when combined with the VC extract and

deep breathing exercise. Unfortunately, this cannot compare to a previous study because of different protocols, VC preparation, age of participants, and severity of nicotine addiction. However, previous data confirmed the benefits of deep breathing exercise in smoking cessation, especially in reducing stress and withdrawal symptoms,[14,41] when compared to the control group without it.[13]

Regarding the oxidative stress results, there were no statistical differences in GSH or MDA in the control group. The GSH level in the rDB/CME with placebo group also did not increase statistically, but MDA was reduced significantly. Whereas both parameters changed significantly in the rDB/CME with VC-product group. In the VC-product group, the mechanism for increasing GSH and reducing MDA can be supported by previous evidence, which proposed VC activity on antioxidant and anti-inflammation.[42] In addition, VC increased GSH, glutathione peroxidase and glutathione S-transferase activity as well as reducing lipid peroxidation in a rat model.[43] However, the MDA level was not only reduced in the placebo product group, but also the product group. That mechanism cannot be explained completely, but it is possible that the effect of honey contained in the placebo product related to an anti-bacteria and anti-inflammatory function, as evidenced previously.[44] Nevertheless, there was no statistical difference in GSH or MDA between the groups after 2 weeks. In addition, this study also calculated the effect size (*dz*) of the MDA result in the rDB/CWE with placebo and product groups, which was the same as the GSH in the rDB/CWE with product group. The statistical significance should be confirmed by an effect size of more than 0.8.[45] This result indicates that a large effect size relates to a significant value in the case of the small sample size in this study.[46] Therefore, this study can conclude the oxidative stress result clinically in the case of a small sample size (n=10).

Finally, unpublished data of the safety profiles from VC used in this study were evaluated clinically such as CBC, and renal and liver function before the program and after 2 weeks of VC-product administration. There was no statistical difference and all of the markers were within normal references. This was similar to previous data that confirmed the safety of VC for smoking cessation at week 8.[47] Therefore, VC is a natural medicinal Thai herb that can be applied for smoking cessation in various forms; for example, jelly candy,[48] lozenges,[49] pastilles,[40] and hard candy.[27] However, the best formula and purification processes of VC and the application protocol must be studied in the future.

CONCLUSION AND LIMITATION

Integration of smoking counseling, relaxed deep breathing, chest mobilization exercise, and hard candy with *Vernonia cinerea* extract can be used for 2 weeks in a smoking cessation program related to oxidative stress status among teenage smokers. However, the short period of 2 weeks and 8 weeks followed-up may not claim to be successful in stopping smoking for a longer period of time. In addition, sample size and other conditions such as age and severity of nicotine addiction should be studied in the future.

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CONFLICTS OF INTEREST

All of the authors and the company confirmed no conflicts of interest in this study.

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