Asian Journal of Chemistry; Vol. 25, No. 14 (2013), 8160-8162

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15425

A Novel Method to Reducing Nicotine Content in Tobacco with Chinese '*Kucai*'

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(*Received*: 5 March 2013; *Accepted*: 5 August 2013) AJC-13895

In order to make a great contribution towards the tar reducing movement and human health promotion all over the world, this study reported a fascinating natural phenomenon and some testing to prove that Chinese '*Kucai*' had the function to reduce nicotine content in tobacco. '*Kucai*' succus, '*Kucai*' powder and '*Kucai*' extract were used to degrade nicotine in tobacco, respectively. The results showed that '*Kucai*' components may be rapidly interacted with nicotine and therefore decrease its concentration. It is hoped that these results can be applied to tobacco industry and health food industry.

Key Words: Chinese '*Kucai*', Degradation, Nicotine content.

INTRODUCTION

A fascinating natural phenomenon has been observed in Chinese farms. If the substance accumulated in a smoking pipe was put into the mouth a snake, the toxic effect was showed almost instantaneously, which was that the snake started to convulse and about to die. At this time, if a few drops of the white succus from the stem of a Chinese herb, named '*Kucai*', whose scientific name is *Ixeris chinensis*, was put into the mouth of the snake, it would resuscitate gradually.

The substance accumulated in the smoking set is tobacco tar and its main component is nicotine. The acute and chronic toxicity of nicotine are well studied. However, there is no specific antidote for acute nicotine poisoning. On the other hand, tobacco smoking has been associated with diseases of the respiratory tract and cardiovascular system[1-3]. On the basis of the observation that *Kucai* can restore a snake from nicotine poisoning, we hypothesized that '*Kucai*' extract may reduce nicotine content in tobacco. Therefore, in this research, experiments were carried out to test if the '*Kucai*' can reduce nicotine content in tobacco.

Kucai or *Ixeris chinensis*, belongs to composites category. (The pictures of '*Kucai*' and tobacco are shown in Fig. 1). Its white succus in stem of this plant contains many components, such as colophony, triterpenoids, flavones, ascorbic acids, amino acids, *etc*.[4-7]. If '*Kucai*' can be improved to reduce nicotine content, it can be applied to tobacco industry and health food industry, which can make a contribution towards the tar reducing movement and human health promotion.

(a) (b)

Fig. 1. Picture of '*Kucai*' and tobacco. (a) Is the picture of '*Kucai*' and its scientific name is *Ixeris chinensis*. This picture was shoot at farm of Agricultural University of Hebei, Baoding, China on October, 2010. (b) Is the picture of tobacco. This picture was shoot at farm in Rong cheng county, Baoding, China on September, 2010

EXPERIMENTAL

Kucai were collected in farm of Agricultural University of Hebei, Baoding, China on October, 2010. Dried and powdered *Kucai* (water content was 7 %), tobacoo were purchased from Rong cheng county, Baoding, China on September, 2010. Nicotine was purchased from wako pure chemical industries, Ltd., Japan.

Experimental design: In order to test if the '*Kucai*' can reduce nicotine content in tobacco, '*Kucai*' succus, '*Kucai*' powder and '*Kucai*' extract were used to degrade nicotine in tobacco, respectively.

Degradation experiment with'*Kucai*' succus: 10 μL of '*Kucai*' succus was added into 50 mL of 0.1 μg mL^{-1} standard nicotine dichloromethane solution and the nicotine content before and after reactions were analyzed through gas chromatography. The nicotine contents were calculated by peak areas through the equation of standard working curve of GC.

Treatment with '*Kucai*' powder on nicotine in tobacco powder: Different amount of '*Kucai*' powder was mixed with 1 g of tobacco powder and extracted with 20 mL of 40 % methanol under sonication at 59 KHz for 45 min. The extract was filtered and its nicotine concentration was determined by gas chromatograph (GC). The nicotine contents were calculated by peak areas through the equation of standard working curve of gas chromatography.

Treatment of nicotine with '*Kucai*' power water extraction solution: 5 g '*Kucai*' powder was added into 100 mL distilled water and dealt with ultrasonic cleaning machine at 59 KHz for 1 h and then was filtrated with filter paper. The filtrated solution reacted with tobacco for 12 h at 60 ºC. The filtrated solution samples before and after reactions were determined by gas chromatograph, respectively.

Determination of nicotine by gas chromatography: A standard solution of nicotine (0.1 μL mL^{-1}) was prepared by dissolving nicotine in dichloromethane. The standard working curve equation of nicotine was confirmed. The analysis was carried out by gas chromatograph, (GC-17A, Shimadzu Corporation, Kyoto). The operating conditions[8] included an injection temperature at 270 ºC and a detector temperature at 270 ºC. Detector gas flow consisted of hydrogen at 50 mL min^{-1}, air at 60 mL min^{-1}. Carrier gas was nitrogen with a constant flow of 50 mL min^{-1}. Injection volume was 2.0 μL with a split ratio of 1:25 and compounds were separated on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DM-1 capillary column. Oven temperature was initially set at 50 ºC and held for 1 min, then increased to 215 ºC at 15 ºC/min and held for 1 min.

Determination of *Kucai* extract or solution by gas chromatography: '*Kucai*' extract or solution was determined by gas chromatography. The operating conditions of GC included an injection temperature at 250 ºC and a detector temperature at 250 ºC. Detector gas flow consisted of hydrogen at 50 mL min^{-1} and air at 60 mL min^{-1}. Carrier gas was nitrogen with a constant flow of 50 mL min^{-1}. Injection volume was 3.0 μL with a split ratio of 50:1. Compounds were separated on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DM-1 capillary column. Oven temperature was initially set at 50 ºC and held for 1 min, then increased to 210 ºC at 10 ºC min^{-1} and held for 1 min.

RESULTS AND DISCUSSION

Effect of '*Kucai*' succus on nicotine: The result of degradation experiment showed that after interact with '*Kucai*' succus, the nicotine content was only 0.067 μg mL^{-1}, which was only 2/3 of the prepared nicotine concentration (0.1 μg mL^{-1}). This indicated that white succus of '*Kucai*' could decrease nicotine content in tobacco.

Effect of '*Kucai*' powder on nicotine content in tobacco: Table-1 showed the effect of different adding amounts of dry '*Kucai*' powders on nicotine content. The adding amounts of dry '*Kucai*' powders were 0 % (control), 5, 10, 15, 20, 25 and 30 % (mass percent content). As shown in Table-1, with the increase of adding '*Kucai*' powder from 0-25 %, the detected nicotine concentration decreased. Further increase of '*Kucai*' powder to 30 % did not significantly change the nicotine content, indicating all the interaction sites of tobacco had been saturated. Adding 25 % of "*Kucai*" powder decreased the nicotine content in tobacco from 43.24 ± 0.47 μg g^{-1} (control) to 20.49 ± 1.01 μg g^{-1} (52.61 ± 0.02 % reduction). This result indicated that under ultrasonic conditions, the dissolved active ingredients in dry '*Kucai*' powder may interact with nicotine and therefore caused the reduction of nicotine content. This test proved that dry '*Kucai*' powder could also reduce nicotine content in tobacco.

Analysis of '*Kucai*' power water extraction solution: In this test, '*Kucai*' power water extraction solution before and after reactions were analyzed by gas chromatography. The GC graphs are shown in Fig. 2. As shown in Fig. 2, dramatic changes were observed on every peak except the first methyl alcohol peak. The chromatographic peaks which had been shown in Fig. 2a at 2.5-15 min were almost disappeared in Fig. 2b. Two new peaks showed up at 2.2 min and 15.9 min, respectively, while none of them corresponds to nicotine peak. These results indicated that '*Kucai*' extract can react with nicotine.

Conclusion

This study provided preliminary evidence that "*Kucai*" components may interact with nicotine and therefore decrease its concentration. Based on these findings; we hypothesized that in the snake case described before, "*Kucai*" components may be rapidly absorbed by snake and interacted with nicotine *in vivo* and therefore deprived nicotine from physiological activities. So, this study can be applied to tobacco industry and health food industry, which can make a contribution towards the tar reducing movement and human health promotion.

Also these results have provided a theoretical basis for further development and utilization of "*Kucai*". However, there are still many questions remain to be answered. For example, what are the active components in "*Kucai*" that interact with nicotine? What are the new substances formed from the interaction? There had no report about this. As a result these questions warrant further investigation.

TABLE-1
EFFECT OF DIFFERENT ADDING AMOUNTS OF DRY '*kucai*' POWDERS ON NICOTINE CONTENT

Adding amounts	0	5 %	10 %	15 %	20 %	25 %	30 %
1	43.04	42.98	41.65	38.43	32.09	21.34	20.05
2	43.78	41.78	39.88	37.67	33.34	20.21	19.78
3	42.89	42.02	41.2	37.12	33.39	19.95	21.64
Average	43.24	42.26	40.91	37.74	32.94	20.5	20.49
SD	0.47648	0.63498	0.919946	0.657799	0.736546	0.738986	1.005037

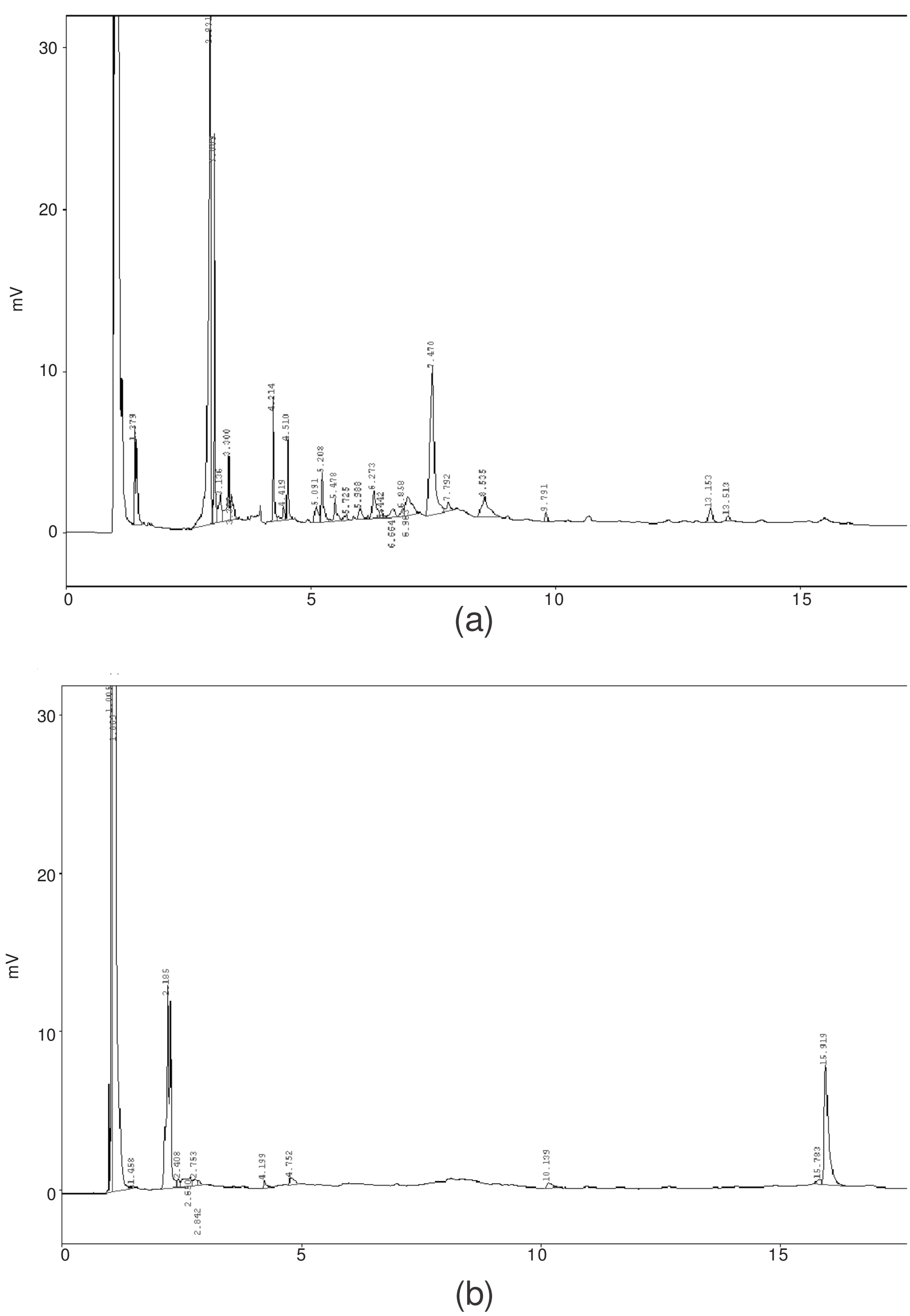

Fig. 2. Gas chromatography of '*Kucai*' extraction solutions. (a) GC of '*Kucai*' solution sample before reacting with tobacco. (b) GC of '*Kucai*' extraction solution after reacting with tobacco

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (NSFC) # 31071580. The authors thank WANG Wenyi for the critical reading of this paper.

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