DETERMINATION OF NICOTINE AND GENERAL TOXICITY OF JORDAN'S MARKET CIGARETTES

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Abstract

Seventeen brands of Jordan market cigarettes were evaluated in terms of nicotine content, percent of nicotine burns during smoking and general toxicity. An accurate, reliable and simple isocratic reverse-phase photo-diode array high performance liquid chromatography (PDA-HPLC) method was adapted. Brine shrimp lethality bioassay (BST) was used to evaluate cigarettes extract toxicity. In general and as predicted, nicotine content of alcoholic water extract of most brands showed higher values than the labeled amount which is usually determined by gas chromatography analysis of smoke condensate. The results show that an average of 69% of cigarette nicotine content either burn or non-volatize during smoking. According to the results obtained, toxicity of cigarettes alcoholic water extract, calculated as LD_{50} against BST, is not proportional to nicotine content; implying other components or additives may contribute to their toxicity.

Introduction

Nicotine is an alkaloid obtained from the leaves of the tobacco plant, *Nicotiana tabacum* Solanaceae. It is a tertiary amine composed of a pyridine and pyrrolidine ring. L-Nicotine is the major tobacco alkaloid and the most pharmacologically active form.[1] Nicotine is highly toxic, carcinogenic, and powerful insecticide. It is recognized as an addictive by such major medical organizations as the Office of U. S. Surgeon, the World Health Organization, the American Medical Association, and the Medical Research in the United Kingdom. It is absorbed into human body by smoking, chewing and snuffing; all of which are extremely hazardous to the human health. Chronic exposure to nicotine may cause an acceleration of coronary disease, peptic ulcer, reproductive disturbances, hypertension, fetal illnesses and birth defects and death. Furthermore, it increases the risk for cancer of various body organs, increase blood pressure and cause vasoconstriction of blood vessels.[2]

The 440 billion cigarettes consumed each year in the United States contain about 2.2 million gallons of nicotine. This chemical is so poisonous that only 40 mg injected

into blood stream can kill a man. Besides nicotine, cigarette smoke consists of a heterogeneous mixture of gases, uncondensed vapors, tar and particulate phase.[3]

Cigarette's smoke harms not only the smoker, but also family members, coworkers, and others who breathe it; called secondhand smoke. In the US about 40,000 non-smokers die each year from diseases directly related to the passive inhalation of secondhand smoke.

Like most other countries, Jordan, 5 million in population, imports and produces several tobacco cigarettes brands. Customers pay more than 283 million dollars yearly. Moreover, the area in which tobacco has been grown in Jordan is about 30,000,000 m^2 from the best fertilized soils. The average annual consuming for each person is about 1680 cigarettes.[4] Several of these products are smuggled to the local market from neighboring countries and sold for cheap prices. These products do not undergo inspection and testing to verify compliance with national regulations.

Various methods to determine nicotine content in different matrices have been reported in the literature including gas chromatography using flame ionization,[5] electron-capture or mass spectroscopyas a detector,[6,7] high performance liquid chromatography (HPLC) with either ultraviolet or mass spectroscopy as a detector,[5,8-15] spectrophotometric,[16] bienzymatic inhibition biosensor,[17] fluorescence emission spectrometry,[18] molecular recognition directed porphyrin chemosensor, and radioimmunoassay.[19,20] In this study, a new liquid extraction procedure and HPLC method using photo-diode array as a detector were developed to determine nicotine content in cigarette's alcoholic water extract. We did not seek a highly sensitive technique, since the concentrations we are dealing with are in the μg/mL level.

An attempt was made to estimate the percentage of nicotine that burn or non-volatize during smoking. This was achieved by calculating the difference between nicotine content in the alcoholic water extract of cigarettes and nicotine concentration in the gas condensate; taken from manufacturer label. In order to investigate whether there is a correlation between nicotine content and general toxicity, seventeen cigarette brands basic alcoholic water extract were examined using brine shrimp lethality test (BST). The brine shrimp (*Artemia salina* leach) is a simple zoologic organism. It is a tool to measure general toxicity or bioactivity in plant extracts. It was developed in 1982 as a

simple, rapid, in-house, bench top, and low cost prescreen for cytotoxicity and pesticidal activities.[21,22]

Results and discussion

In this work, we were trying to answer the following questions: is nicotine content of alcoholic water extract of cigarette significantly different from the labeled gas condensate value, what is the percentage of nicotine that either burn or non-volatize during smoking, and is nicotine primarily responsible for the general toxicity of cigarettes' basic alcoholic water extract. A method of nicotine extraction from cigarette was developed. Through testing several extraction schemes, results suggest that basic alcoholic mixture gave high recovery in shorter time and less tedious work than acidic alcoholic mixtures or pure methanol. A modified isocratic reverse-phase HPLC method was used which provided an accurate, judging from quality control (QC) samples and reproducible results. A chromatogram of a cigarette extract is presented in Figure 1.

Table 1. Nicotine content of Jordan's tobacco cigarettes and the percent burns during smoking.

Tobacco Product	Nicotine Content[a], mg/cigarette		
	Liquid Extract[a]	Gas Condensate[b]	% Difference[c]
Global Products			
Marlboro Light®	3.66	0.8	78
Marlboro®	6.13	0.9	85
Rothmans®	4.06	1.0	75
Kent Light®	2.28	0.7	69
Kent®	3.03	0.9	70
Winston®	1.77	0.9	49
Winston Light®	0.82	0.7	15
Local Products			
Nova®	1.88	0.8	57
Viceroy®	10.15	0.6	94
Palace®	5.30	1.0	81
Palace Light®	2.24	0.8	64
Classic®	3.89	1.0	74
Kareem®	15.30	1.0	94
Mond Light®	0.72	0.4	44
Goldstar®	3.23	0.8	75
Hamawi®	16.88	NA[d]	-
Rum Light®	3.13	0.6	81

[a] Nicotine concentration of basic alcoholic water extract of tobacco cigarettes.
[b] Values are taken from the manufacturer label.
[c] Difference % = [liquid extract – gas condensate] / [liquid extract] × 100.
[d] No value was reported on the label.

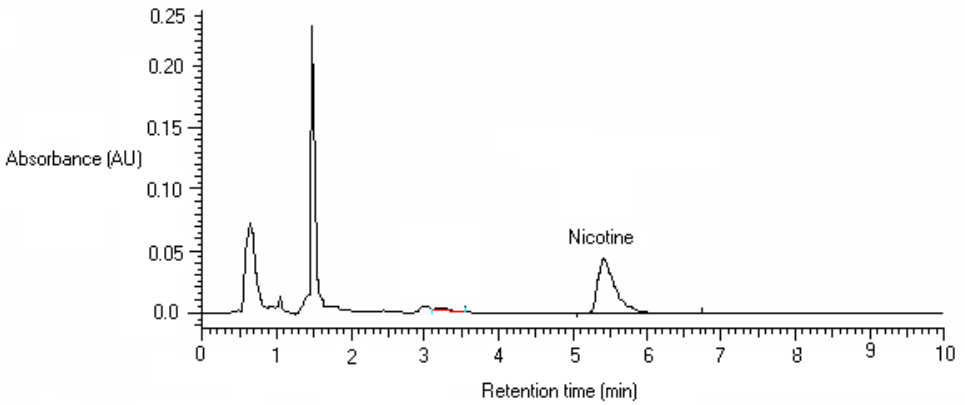

Figure 1. HPLC chromatogram of nicotine in cigarette extract.

Contents of nicotine in 17 cigarette brands sold in Jordan in 2002 are different. Also, they are differences between their gas condensate values (Table 1). Nicotine contents in all cigarette brands are higher than the labeled amounts which are usually determined by gas chromatographic analysis of smoke condensate.[23] This is predictable since nicotine is a volatile substance that either burns or non-volatizes during smoking; the temperature of the tip of cigarette may reach 600-800 °C. The results show that an average of 69% (15-94) of cigarette nicotine content either burns or non-volatizes during smoking.

Jordan market cigarettes were different in their general toxicity. Brine shrimp lethality test (Table 2) showed that nicotine is the major contributor, but not the only, to the general toxicity of cigarettes' basic alcoholic extract; other compounds or additives may contribute in this regard.

We expected that cigarettes with lower nicotine content having higher LD_{50} values, means relatively "safer". This was not true for most products. For example, Winston® with 1.77 mg of nicotine/cigarette gave an LD_{50} of >1000 ppm, considered relatively "safe", where as Nova® with 1.88 mg of nicotine/cigarette gave an LD_{50} of 111 ppm. The differences in nicotine content can not cause the large difference in their LD_{50}s. Several light products showed higher toxicity than their corresponding heavy product (Table 2), for example Winston® versus Winston® light and Palace® versus Palace light®.

Based on this study, most local cigarette brands showed lower LD_{50}, means more toxic, than most global brands; sometimes even with comparable nicotine content (Table

1 and Table 2). This might be due to botanical and agronomical factors, plantation environment, manufacturing processes, storage conditions, and other contaminants or additives.

Table 2. Brine shrimp lethality test of Jordan's tobacco products.

	LD_{50} in ppm (μg/mL)
Nicotine	5.9
Global Products	
Marlboro Light®	> 1000
Marlboro®	> 1000
Rothmans®	218
Kent Light®	565
Kent®	310
Winston®	> 1000
Winston Light®	126
Local Products	
Nova®	111
Viceroy®	58
Palace®	219
Palace Light®	50
Classic®	114
Kareem®	289
Mond Light®	146
Goldstar®	108
Hamawi®	145
Rum Light®	199

Conclusions

HPLC method and a simple bioassay were adapted to test seventeen brands of tobacco cigarettes. Our results showed that an average of 69% of nicotine content either burns or non-volatizes during smoking. The toxicity of cigarettes' basic alcoholic water extract, calculated as LD_{50} against Brine shrimp lethality test (BST), is not proportional to nicotine content; suggesting other components or additives may contribute to their toxicity. The percent of nicotine burned may become an important factor in adjusting nicotine content of tobacco; thus controlling its toxicity. We would suggest further and more rigorous *in vitro* and *in vivo* clinical study to see if this *in vitro* data will correlat with an *in vivo* data.

Experimental

Tobacco Extraction

A seventeen different types of local and worldwide brand cigarettes (light and heavy) sold in Jordan in 2002 were collected randomly from different places in Jordan with different batch numbers (Table 1 and Table 2). A weight of twelve cigarettes of each type using four digits analytical balance (Sartorius® analytic model A 120 S) was recorded. The following liquid extraction procedure scheme was followed: 12 cigarettes from different batches were weighed, homogenized and soaked in a mixture of 25% NH_3:H_2O:methanol with a ratio of 2:1:1. The extract was shaken for 48 hours using an automated shaker (model D-3162 Kottermann labortechnik, type 3047, West Germany); this step was repeated three times. The extraction solution was combined and filtered using a filter paper. The aqueous solution was extracted three times with 50 mL of dichloromethane in a separatory funnel. The organic layer was brought to dryness in a vacuum hood. Complete recovery was checked using thin-layer chromatography. The residue was redissolved in 50 mL of deionized water.

HPLC Analysis

A simple isocratic reverse-phase HPLC method with external standard was used. The HPLC consisted of LaChrom® Merck equipped with quaternary gradient L-7150 pump, Superspher 100 125-4 C_{18} column, and L-7455 PDA detector. The mobile phase used was a mixture of acetonitrile and 0.10 M NH_3 in water (30:70 v/v). UV maximum absorption for nicotine was observed at 254 nm. Flow rate was set at 1.0 mL min^{-1}. Aliquots of 20 μL were injected into HPLC column. Calibration curve was constructed by preparing a 1000 μg mL^{-1} stock solution of nicotine (50 mg of nicotine in 50 mL deionized water). Concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg mL^{-1} were prepared by micro-pipetting from the stock solution. Calibration curve, measuring peak area, was linear over the working range from 0.5 to 100.0 μg mL^{-1} with r^2 value obtained > 0.9986. Two replicates of each type of cigarette extract were auto-injected into HPLC. Results showed that precision of nicotine analysis of all samples is acceptable; RSD was less than 5.0%.

Reagents

Nicotine (BDH chemicals, Poole, UK), HPLC grade methanol and acetonitrile (Scharlau, Spain).

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References and Notes

1. B. R. Martin, M. D. Aceto, *Neurosci. Biobehav. Rev.* **1981**, *5*, 473–478.
2. K. A. Perkins, N. Benowitz, J. Henningfield, P. Newhouse, O. Pomerleau, G. Swan, *Addiction*, **1996**, *91*, 129–144.
3. L. A. Mooney, R. M. Santella, L. Covey, A. M. Jeffrey, W. Bigbee, M. C. Randall, T. B. Cooper, R. Ottman, W.-Y. Tsai, L. Wazneh, A. H. Glassman, T.-L. Young, F. P. Perera, *Cancer Epidemiol. Biomarkers Prev.* **1995**, *4*, 627–634.
4. UNICEF with Cooperation of the Ministry of Health and the Ministry of Education, New Generations without Smoking Project, UNICEF Office **2002**, Amman, Jordan.
5. K. Tyrpien, T. Wielkoszynski, B. Janoszka, C. Dobosz, D. Bodzek, Z. Steplewski, *J. Chromatogr. A*. **2000**, *870*, 29–38.
6. E. Davoli, L. Stramare, R. Fanelli, L. Diomede, M. Salmona, *J. Chromatogr. B*. **1998**, *707*, 312–316.
7. H. James, Y. Tizabi, R. Taylor, *J. Chromatogr. B*. **1998**, *708*, 87–93.
8. M. Hariharan, T. VanNoord, *Clin. Chem*. **1991**, *37*, 1276–1280.
9. J. A. Saunders, *Drug Inf. J.* **1998**, *32*, 609–617.
10. M. Nakajima, T. Yamamoto, Y. Kuroiwa, T. Yokoi, *J. Chromatogr. B*. **2000**, *742*, 211–215.
11. H.-S. Shin, J.-G. Kim, Y.-J. Shin, S. H. Jee, *J. Chromatogr. B*. **2002**, *769*, 177–183.
12. G. N. Mahoney, W. Al-Delaimy, *J. Chromatogr. B*. **2001**, *753*, 179–187.
13. R. Pacifici, S. Pichini, I. Altieri, M. Rosa, A. Bacosi, A. Caronna, P. Zuccaro, *J. Chromatogr. B.* **1993**, *612*, 209–213.
14. P. Zuccaro, I. Altieri, M. Rosa, A. R. Passa, S. Pichini, G. Ricciarello, R. Pacifici, *J. Chromatogr. B.* **1993**, *621*, 257–261.
15. G. Stehlik, J. Kainzbauer, H. Tausch, O. Richter, *J. Chromatogr*. **1982**, *232*, 295–303.
16. S. A. Al-Tamrah, *Anal. Chim. Acta*. **1999**, *379*, 75–80.
17. L. Campanella, R. Cocco, G. Favero, MP Sammartino, M. Tomassetti, *Ann. Chim.* **2002**, *92*, 373–385.
18. G. R. Deviprasad, F. D'Souza, *Chem. Commun.* **2000**, 1915–1916.
19. N. J. Haley, D. Hoffmann, *Clin. Chem*. **1985**, *31*, 1598–1600.
20. J. Klein, R. Forman, C. Eliopoulos, G. Koren, *Ther. Drug Moint*. **1994**, *16*, 67–70.
21. J. L. McLaughlin, N. R. Ferrigni, *Processing of Symposium on Drug Discovery and Development of Naturally Occurring Anti-tumor Agents*, NCI/FCRF, Frederick, MD, **1982**, *27–29*, 9–12.
22. J. L. McLaughlin, *Methods in Plant Biochemistry*, K. Hostettmann ed. Academic press, London, **1991**, *6*, 1–32.
23. International Standard ISO 10315:2000, Technical Corrigendum 1, 11/15/**2000**.

Povzetek

Določevali smo vsebnost nikotina in toksičnost cigaret dostopnih na trgu v Jordaniji. Nikotin smo določevali s tekočinsko kromatografijo, za določanje strupenosti smo uporabljali morske rake (BST). Ugotovili smo, da so vsebnosti nikotina bistveno višje kot je označeno na cigaretah. Razlike so posledica različne priprave vzorcev. Podatki o vsebnosti nikotina, ki so označeni na cigaretah so rezultati analiz kondenzata po sežigu cigaret, medtem ko smo mi določali nikotin v ekstraktih cigaret. Naši rezultati so približno dvakrat višji. Test strupenosti je pokazal, da letalna doza (LD_{50}) ni proporcionalna vsebnosti nikotina. Torej k strupenosti prispevajo še druge spojine, ki so prisotne v cigaretah.