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Antimicrobial and cytotoxicity potential of *Peganum harmala* smoke

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ABSTRACT

Peganum harmala seeds' smoke is traditionally used in Iran as both a disinfectant agent and for all kinds of rituals against evil eye and bad luck. Although a report have been published in the literature on the antimicrobial activity of dichloromethane condensate prepared from *P. harmala* seeds (Esphand), the ability of the smoke to reduce microorganisms in air has yet to be understood. In this research the antimicrobial activity of *P. harmala* smoke on the surface culture of different microorganisms has been studied. Also the present study's aim was to determine the cytotoxic potential of *P. harmala* and its smoke condensate using a standardized exposure protocol in the well established cell line, Balb/C 3T3 fibroblasts. The smoke was found to be effective against all tested organisms. Fungi appear to be more sensitive than bacteria. Also the samples (smoke condensate and methanol extract) demonstrated a wide range of cytotoxicity against the 3T3 cells, with the smoke condensate being the most potent. Only 4.8 µg/ml smoke condensate was necessary to decrease cell proliferation by 50%. To obtain the same result required a noticeably higher concentration (63.1µg/ml) of *P. harmala* seeds' methanol extract.

KEYWORDS: antimicrobial activity, cytotoxicity, seeds, *Peganum harmala*, smoke, air disinfectant.

INTRODUCTION

Peganum harmala L. is a wild-growing flowering plant belonging to the Zygophyllaceae family and is found abundantly in the Middle East and North Africa (1). *Peganum harmala* seeds are commonly named "Esphand" in Iran. Since ancient times it has been considered an important medicinal plant. The *P. harmala* seeds are known to possess hypothermic and hallucinogenic properties (2,3). It has traditionally been used as an abortifacient agent in the Middle East and North Africa (4). Several reports in the literature indicate a great variety of pharmacological activities of *P. harmala* such as antimicrobial, antitumor, antinociceptive and MAO-inhibiting activities (5-7). Its smoke is widely used in Iran and Turkey for all kinds of rituals against evil eye and bad luck, and as a disinfecting agent (8).

Recently the chemical composition and antimicrobial activities of dichloromethane condensate prepared from *P. harmala* seeds have been reported. The Harmine has been identified as *P. harmala* smoke's active component (9). The chemicals reported to be present in this smoke preparation are found in Table 1 (10). However, the air disinfection potential and cytotoxicity property of this smoke have yet to be unknown. In this research the antimicrobial activity of *P. harmala* smoke on the surface culture of different microorganisms (on the interface between gaseous phase and agar medium) has been studied. Also other objective of this investigation was to determine the cytotoxicity of *P. harmala* seeds' methanol extract, and its smoke condensate on Balb/C 3T3 fibroblast cells. The determination of an agent's intrinsic cytotoxicity is becoming an important

component characterizing its toxicological properties (11).

MATERIALS AND METHODS

Plant materials

Peganum harmala L. (Zygophyllaceae) seeds were collected from Abyaneh (Isfahan Province), Iran in June 2003, and were authenticated by Dr. H. R. Monsef-Esfahani. Voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Antimicrobial assay

In this study, antimicrobial activity of *P. harmala* smoke was investigated using the following method. Different microorganisms (listed in Table 1) were used as test strain. The seeds (1, 2, 3 and 4 g) were burned on the hot surface of a heater in a sealed cabinet room (50 × 50 × 50 cm) for 1 min. Subsequently, the inoculated plates (10^4 cells per plate) were located in the cabinet and treated with the smoke for 15 min. Moreover a series of inoculated plates without smoke treatment were used as control. All plates were incubated at the conventional conditions for the growth of fungi (24h at 25^{o}C for yeasts and 48h at 25 ^{o}C for filamentous fungi), and bacteria (24h at 37 ^{o}C). After incubation periods, the plates were observed and number of colonies was counted.

Extraction and preparation of smoke condensate

The dried *P. harmala* seeds were pulverized and extracted three times by maceration in methanol for 24 hours at room temperature. To manufacture the smoke extracts, smoke from smoldering seeds (100 g) was passed to a condensing tower where it was captured in dichloromethane. The combined methanol extracts and smoke condensate were evaporated to yield a dark-brown viscous residue, and were reserved for cytotoxicity experiments. All experiments were performed based on the concentrated extracts' dry mass.

Cell culture

The Balb/C 3T3 fibroblast cells were routinely grown in a medium comprised of 90% Dulbecco's modified Eagle's medium (DMEM), 5% fetal calf serum (FCS), 5% newborn calf serum (NBCS), 2 mM glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin (referred to as growth medium). The cultures were maintained in a 5% CO_2 incubator in a humidified atmosphere at 37°C. The 3T3 cells were obtained from the National Cell Bank of Iran (NCBI), Pasteur Institute of Iran, Tehran, Iran.

Dose-response analysis

The cells were grown to confluence in 175 cm^2 flasks and were then removed from the flasks by incubation at 37 °C with a trypsin/EDTA solution for 5–10 min. The cells were then added to 96 well cell culture plates at a density of 20,000 cells per well (100 µl). The cells then were allowed to attach for 24 h prior to exposure to the tested samples. Triplicate, twofold dilutions of methanol extract of Esphand and dichloromethane condensate were transferred to the cultured cells. Non-treated cells were used as a control. The cultures were maintained in a 5% CO_2 incubator at 37°C and were examined daily under a microscope. Cell counts were taken of 0.1-ml samples taken from cultures after vigorous shaking, to monitor cell viability using the conventional trypan blue colorimetric method (12). Cytotoxicity was expressed as the percentage of viable cells at different sample concentrations. IC50 was calculated as the concentration of testing extracts that decrease 50% of the cell number compared to that of the control group without extracts.

RESULTS AND DISCUSSION

Although a report have been published in the literature on the antimicrobial activity of dichloromethane condensate prepared from *P. harmala* seeds (Esphand), the ability of the smoke to reduce microorganism in air has yet to be understood (9). The data presented in Table 1 indicate that the smoke generated from burning *P. harmala* seeds reduced the viability of tested microorganisms. No colonies were detected on the surface of the plates treated by smoke generated from 4g burned seeds. Fungi appear to be more sensitive than bacteria and these finding confirm our previously published data (9). The cytotoxicity of the *P. harmala* and its smoke condensate was evaluated *in vitro* against Balb/C 3T3 fibroblasts at different concentrations. Our cytotoxicity analysis of the samples shows a direct dose-response relationship; cytotoxicity increased at higher concentrations (Figure 1, 2). The samples demonstrated a wide range of cytotoxicity against the 3T3 cells, with the smoke condensate being the most potent. The concentration necessary to produce 50% cell death was 4.8µg/ml for the smoke condensate, while approximately a 63.1 µg/ml concentration of the *P. harmala* seeds' methanol extract produced the same effect (Table 2). As shown in Figure 1, the presence of 80 µg/ml of *P. harmala* total extract significantly inhibited the cell line's growth, while lower dosage levels (10, 20 µg/ml) showed no cytotoxicity on the tested cell line. In contrast, the cells' proliferation was greatly inhibited at concentrations above 4 µg/ml of the smoke preparation. At 8 µg/ ml, the smoke condensate was able to inhibit the cell line's growth by more than 95%. A previous study of the antimicrobial activity of the two condensates prepared from *P. harmala* smoke established that harmine was required to kill bacteria

Table 1: Antimicrobial activity of the smoke from P. harmala seeds [a].

Micro-organisms	Number of colonies				
		1g	2g	3g	4g
S. aureus (ATCC 25923)	N	505	160	-	-
S. epidermidis (ATCC 12228)	N	630	350	-	-
E. coli (ATCC 25922)	N	N	485	150	-
P. aeruginosa (ATCC 27853)	N	543	240	-	-
C. neoformans (PLM 589)	N	40	13	-	-
A. fumigatus (PLM 112)	N	33	<10	-	-
A. niger (PLM 16404)	N	N	36	<10	-

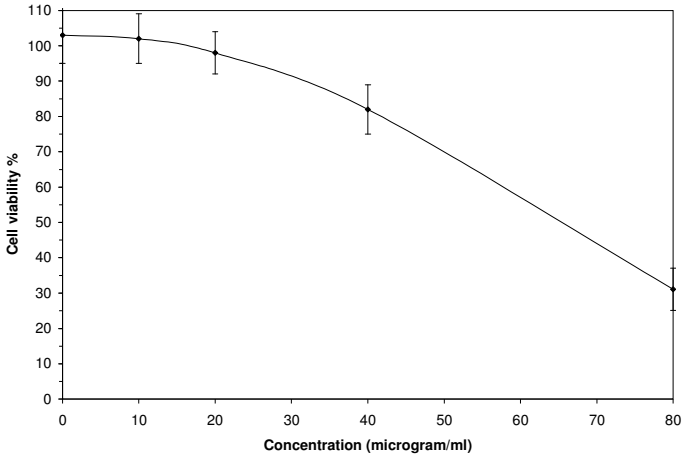
N, non-countable

[a]All counts were done in triplicate.

Table 2: Constituents of Peganum harmala smoke condensate in dichloromethane (10).

No.	Constituents	Concentration %
1	Phenol	0.24
2	2-Ethyl hexanol	1.22
3	p-Cresol	0.25
4	Nonan-1-ol	0.31
5	Phenol-2,6- dimethyl	0.25
6	Cyclohexan , nitro	0.17
7	Quinazoline	0.18
8	Indole	0.22
9	Skatole	0.3
10	Tetradecane	1.55
11	Allyl decanoate	0.41
12	Pentadecan	1.25
13	Tridecanol	0.33
14	Hexadecane	2.90
15	Tetradecanol	1.88
16	2-Octanol benzoate	3.09
17	Heptadecane	6.16
18	Pentadecane- 2,6,10,14 -tetramethyl	2.60
19	Dodecanoic acid butylester	0.65
20	Octadecane	7.57
21	Hexadecane 2,6,10,14 tetramethyl	3.65
22	Hexadeconol	1.53
23	Nonadecane	8.12
24	Dibutyl phthalate	3.6
25	Eicosane	9.28

26	Octadecanol	1.5
27	Methyl oleate	1.5
28	Henicosane	12.91
29	Harmine	14.15
30	Tricosane	5.05
Total	Known components	92.82



Figiure 1. The cytotoxicity of the Peganum harmala seeds' methanol extract on the Balb/C 3T3 fibroblast cells.

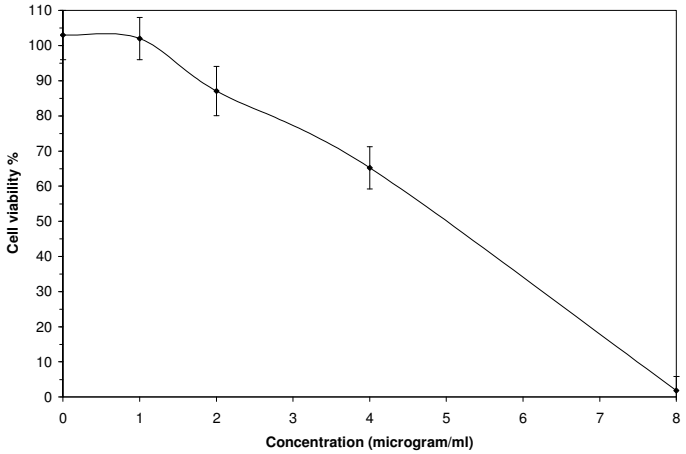


Figure 2: The cytotoxicity of the Peganum harmala smoke's dichloromethane preparation on the Balb/C 3T3 fibroblast cells.

and fungi. In this study we tested the cytotoxic potential of *P. harmala* and its dichloromethane smoke extract using a standardized exposure protocol in the well established cell line, Balb/C 3T3 fibroblasts. The samples demonstrated a wide range of cytotoxicity against the 3T3 cells, with the smoke condensate being the most potent (IC50 = 4.8 µg/ml). It seems that the Esphand smoke preparation's higher cytotoxicity against 3T3 cells cannot be related to harmine. Harmine has been detected in *P. harmala* seeds and in its smoldering smoke (9, 10). Other major or minor components such as henicosane or alkyldecanes, detected in the dichloromethane extract of *P. harmala* , may be responsible for this strong cytotoxicity. Extracts of *P. harmala* have been reported to exhibit cytotoxicity against various cell lines (13). No studies have been

conducted, however, on the cytotoxicity of smoke preparation from *P. harmala* seeds. This is the first study of the cytotoxicity of smoke condensate from *P. harmala* seeds. This study demonstrates antimicrobial activity of *P. harmala* smoke which parallels the traditional use of *P. harmala* smoke as a disinfectant agent.

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