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Cytotoxicity and Antimicrobial Activity of Harman Alkaloids

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Abstract: The cytotoxicity and antimicrobial activities of harman alkaloids including harmane, harmine, harmalol and harmaline were investigated. The cytotoxicity was monitored by the brine shrimp lethality test and microdilution method was used to determine MIC and MBC of the compounds. Harmane showed the most cytotoxicity and the most antimicrobial activity.

Key words: Harman alkaloids, cytotoxicity, MIC, MBC

INTRODUCTION

The harman alkaloids are a group of beta-carboline alkaloids that present in some medicinal plants such as *Peganum harmala* L. (syrial rue) and *Banisteriopsis caapi* L. (yage). These plants have been used for the treatment of various diseases including cancers and malaria in oriental traditional medicine for 2 millennia (Nadikarni, 1976). A number of pharmacological effects have been attributed to such alkaloids such as tremorogenesis (Louis *et al*., 2002), hypothermia (Fattah *et al*., 1995), hallucinogenesis (Grella *et al*., 1998) monoamine oxidase inhibition (May *et al*., 1991; Rommelspacher *et al*., 1994). They are also known to bind to receptors like 5-HT receptors and the benzodiazepine binding site of GABA receptors (Saano *et al*., 1982). The spasmolytic effects of harmane, harmine and harmaline have also been reported on guinea-pig isolated trachea (Shi *et al*., 2001). In addition, these compounds possess antioxidative and radical scavenging properties (Tse *et al*., 1991; Herraiz *et al*., 2002) inhibition of platelet aggregation (Saeed *et al*., 1993), cytotoxic effects on cancer cell lines and inhibition of DNA topoisomerase I (Sobhani *et al*., 2002). Reports are also available on cardiovascular actions of these alkaloids (Shi *et al*., 2000) and the vasorelaxant effects of harmane, harmine, harmaline and harmalol seem to contribute to their hypotensive effects (Shi *et al*., 2001). Anthelmentic and antiprotozoal activities have also been attributed to harman alkaloids (Ancolio *et al*., 2002).

In the present study, we evaluated the cytotoxicity and antimicrobial activity of harman alkaloids including harmane, harmine, harmalol and harmaline.

MATERIALS AND METHODS

Materials

Harman alkaloids including harmane, harmine, harmalol and harmaline were obtained from Fluka Chemical Co. and used without further purification.

Antimicrobial Assay

The following strains were used as test organism: *Staphylococcus aureus*, *Escherchia coli*, *Proteus vulgaris* and *Candida albicans*. The Minimum Inhibitory Concentration (MIC) and Minimum

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Bactericidal Concentration (MBC) of all alkaloids were determined by microdilution method in Mueller- Hinton broth (Difco) for bacteria and the Sabourad medium for *Candida albicans*. Inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard [10^8 colony-forming units (CFU) mL^{-1}] and diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37°C and the MICs were recorded after 24 h of incubation for bacteria and after 48 h of incubation for *Candida albicans*. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of compounds at which the microorganism tested did not demonstrate visible growth. Minimum Bacteriostatic Concentration (MBC) was defined as the lowest concentration yielding negative subcultures or only one colony.

Brine Shrimp Bioassay

The brine shrimp lethality test was used. In brief, brine shrimp (*Artemia salina*) eggs were hatched in artificial seawater (38 g L^{-1} of sea salt). After 48 h of incubation, ten brine shrimps were transferred to each sample vial using a pasteur pipette and artificial sea water was added to make 5 mL. Sample vials were previously prepared by dissolving 10 mg of each compound in 1 mL of DMSO. From this solution 5, 10, 15, 30 and 50 μL was transferred to vials corresponding to 10, 20, 30, 60 and 100 μg mL^{-1}. The solvent was then evaporated overnight. Survivors were counted after 24 h and the LC_{50} values, with 95% confidence intervals were determined using probit analysis. Control vials were prepared using DMSO only. Three replicates were prepared for each dose level.

RESULTS

Antimicrobial

The MIC and MBC values of these compounds show the following order of potency for *Staphylococcus aureus*, *Escherchia coli* and *Proteus vulgaris* inhibition: harmane>harmine>harmaline>harmalol and following order of potency for *Candida albicans* inhibition: harmane>harmaline>harmalol>harmine (Table 1 and 2).

Cytotoxicity

It is revealed from the Table 3 that all the compounds tested in this study, caused brine shrimp death compared with other cytotoxic natural compounds for example berberin chloride

Table 1: Minimum inhibitory concentration (MIC mg mL^{-1}) of harman alkaloids

Test organism	Harmaline	Harmine	Harmalol	Harmane
S. aureus	0.9	0.6	1.2	2.0
E. coli	2.5	0.7	2.0	0.6
P. vulgaris	2.0	2.5	5.0	0.6
C. albicans	0.2	0.6	0.4	0.1

Table 2: Minimum bactericidal concentration (MBC mg mL^{-1}) of harman alkaloids

Test organism	Harmaline	Harmine	Harmalol	Harmane
S. aureus	1.0	0.7	1.5	2.5
E. coli	2.7	0.8	2.5	0.7
P. vulgaris	2.1	3.0	8.0	0.7
C. albicans	0.4	0.7	0.5	0.2

Table 3: Cytotoxicity of harman alkaloids

Tested compound	LC_{50} (μg mL^{-1})	95% confidence limits (μg mL^{-1})	Regression equation
Harmane	50.00	27.88-63.75	y = 1.16+2.87x
Harmine	23.74	14.85-31.75	y = 0.97+0.53x
Harmalol	09.88	01.19-24.49	y = 1.50+0.20x
Harmaline	23.00	18.06-37.33	y = 0.48+1.60x

(LC_{50} = 22.5 μg mL^{-1}) and strychnine sulfate (LC_{50} = 77.2s μg mL^{-1}) (Meyer *et al*., 1982). Harmine showed the most cytotoxicity (LC_{50} = 9.88 μg mL^{-1}) followed by harmane (LC_{50} = 23 μg mL^{-1}), harmaline (LC_{50} = 23.74 μg mL^{-1}) and harmalol (LC_{50} = 50 μg mL^{-1}).

DISCUSSION

The results of antimicrobial investigation confirmed the antibacterial and antifungal activity of harman alkaloids. Due to their strong toxicity and their possible genotoxicity in mammalian cells, harman alkaloids can not represent interesting therapeutic antibacterial and antifungal agents, however their presence in several medicinal plants might contribute to the prevention of bacterial and fungal infections in the populations using traditional medicine.

The cytotoxicity of plant materials is considered to be due to the presence of antitomour compounds. Harman alkaloids including compounds tested in this study are major compounds in some medicinal plants such as peganum harmala and the results of this study demonstrates that these compounds have probably defense role in this plants. They showed very good cytotoxicity and more study are needed to demonstrate usefulness of them. These compounds with their analogues can be new anticancer drugs in the future and harman alkaloids containing plants will be a good source for them.

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