Indian J Med Res 129, May 2009, pp 566-568

In vitro interference of *Hyptis martiusii* Benth. & chlorpromazine against an aminoglycoside - resistant *Escherichia coli*

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Received January 31, 2008

The antibacterial and synergistic activity of the ethanol extract from *Hyptis martiusii* Benth. was assayed by microdillution. The growth of two isolates of *Escherichia coli* tested was inhibited by the extract. The minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) values ranged from 512 and >1024 μg/ml for the *E. coli* 27 and 1024 and > 1024 μg/ml for the *E. coli* ATCC8539, respectively. A synergism between this extract and all aminoglycosides assayed was demonstrated. In the same form synergism between chlorpromazine and kanamycin, amikacin and tobramycin was observed, indicating the involvement of an efflux system. Extracts from *H. martiusii* could be used as a source of plant derived natural products with modifying antibiotic activity and these products may interact and affect multidrug resistance systems (MDR) as efflux pumps.

Key words Aminoglycosides - antibiotic activity - *Hyptis martiusii* - multidrug resistance – synergism

With increased incidence of resistance to antibiotics, natural products from plants could be interesting alternatives[1,2]. Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy[3]. Many plants have been evaluated not only for direct antimicrobial activity, but also as a resistance-modifying agent[4].

Hyptis martiusii Benth (cidreira-do-campo) is a small shrub belonging to family *Labiatae* with a few pharmacological reports. Antitumoural, cytotoxic and insecticidal activities were identified[5,6], but no antimicrobial activity has so far been reported. Several chemical compounds such as flavonoids[7], diterpenes[8] e sesquiterpenes[9], with several biological and pharmacological activities[10], have been isolated from other plants of the genus *Hyptis.*

Aminoglycosides are potent bactericidal antibiotics targeting the bacterial ribosome, and the increase in cases of bacterial resistance to aminoglycosides is widely recognized as a serious health threat[11]. In

Escherichia coli, the main mechanisms of resistance to aminoglycosides are active efflux and enzymatic inactivation[12]. In this study, we tested an ethanol extract of *H. martiusii* and chlorpromazine as a resistance modifying agent in an aminoglycoside-resistant isolate of *E. coli*.

The experiments were performed in the Laboratory of Genetic of Microorganisms (Department of Molecular Biology) in the Federal University of Paraíba, Brazil. The experiments were performed with clinical *E. coli* isolate (EC27) resistant to neomycin and gentamicin (low level) and to tobramycin, amikacin and kanamycin[13]. The EC-ATCC8539 strain of *E. coli* was used as a positive control. All strains were maintained in heart infusion agar slants (HIA, Difco, USA), and prior to assay, the cells were grown overnight at 37ºC in brain heart infusion (BHI, Difco, USA). Leaves of *H. martiusii* were collected in the county of Crato, Ceará State, Brazil. The plant material was identified by Dr Arlene Pessoa and a voucher specimen was deposited with the number 464 at the Herbarium "Dárdano de Andrade Lima" of Universidade Regional do Cariri - URCA. The drugs used (chlorpromazine, gentamicin, tobramycin, kanamycin, amikacin and neomycin) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All drugs were dissolved in sterile water.

Aerial parts 300 g were dried at room temperature and powdered. The powdered material was extracted by maceration using 1 l of 95 per cent ethanol as solvent at room temperature, and the homogenate was allowed to stand for 72 h at room temperature. The extracts were then filtered and concentrated under vacuum in a rotary evaporator (mod. Q-344B - Quimis, Brazil) and ultrathermic bath (mod. Q-214M2 - Quimis, Brazil)[14]. Each 200 g of aerial parts yielded 5-6 g of ethanol extract of *H. martiusii* (EEHM). For the tests, the dry extract material was dissolved in DMSO.

The minimum inhibitory concentration (MIC) of EEHM, antibiotics and chlorpromazine (CPZ) were determined in BHI by the microdilution assay using suspensions of 10^5 cfu/ml and a drug concentration range of 1024 to 1 mg/ml (two-fold serial dilutions)[15]. MIC was defined as the lowest concentration at which no growth was observed. For the evaluation of EEHM as a modulator of antibiotic resistance and the possible interference with efflux systems, MICs of the antibiotics were determined in the presence of the extract (EEHM - 32 μg/ml) and in the presence of efflux pump inhibitor (CPZ - 16 μg/ml) at sub-inhibitory concentrations. The plates were incubated for 24 h at 37ºC. CPZ was used as positive control for efflux pump inhibition.

The EEHM showed antibacterial activity at 1024 μg/ml against the strains assayed (MIC >1024 μg/ml). Besides the antibacterial activity of EEHM, when it was added to the growth medium at 32 μg/ml, a dramatic reduction in the MIC for all aminoglycosides was observed in *E. coli* 27 (but not with ATCC 8539, possibly due the absence of any resistance mechanisms to aminoglycosides - data not shown), demonstrating a synergistic effect of EEHM on aminoglycoside activity (Table). As far as we know, no synergism between natural products of *H. martiusii* and aminoglycosides has been reported. Reduction in MIC for kanamicin, tobramicin and amikacin was also observed when CPZ was incorporated into the growth medium at 16 μg/ml, indicating that the EEHM probably acted as a putative inhibitor of an efflux pump (Table).

Phenothiazines, such as chlorpromazine, probably act on the plasma membrane of bacteria affecting the efflux pumps[16]. This modification of permeability could enhance the activity of antibiotics that act within the cell, such as the aminoglycosides. A synergistic effect of CPZ on gentamicin or neomycin was not observed, which suggested the occurrence of other resistance mechanisms or of a CPZ insensitive efflux pump that can be blocked by EEHM in the case of gentamicin (Table).

The results obtained have indicated that *H. martiusii* (and broadly Lamiaceae) could serve as a source of plant derived natural products with a property of modifying antibiotic activity, an interesting alternative to combat the antibiotic multiresistance.

Table. Minimum inhibitory concentration (MIC) values (μg/ml) of aminoglycosides in the absence and presence of EEHM and CPZ in *E. coli* 27

	EC 27		
	MIC	MIC combined	
Antibiotics		EEHM (32 μg/ml)	CPZ (16 μg/ml)
Amikacin	64	≤ 1	16
Gentamicin	8	≤ 1	8
Kanamycin	256	2	8
Neomycin	16	3	16
Tobramycin	32	≤ 1	≤ 1
Chlorpromazine	64	-	-

EEHM, Ethanolic extract of *H. martiusii*
CPZ, chlorpromazine

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