

An Exploratory Study on the Antimicrobial Activity of Cetirizine Dihydrochloride

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Antihistamines belong to H1 receptor antagonist class of drugs. Pharmacologically they are classified into two categories namely first generation, mostly sedating in nature and second generation, which are less sedating and quite selective in activity. Widespread uses of antihistamines were found during microbial infections of various types. The utmost deliberate effort in comprehending the actions of antihistamines within the extent of antimicrobials forms the basis of the investigation. The antimicrobial activity of antihistamines explored by previous researchers helped in determining the minimum inhibitory concentration of cetirizine against 51 strains of bacteria. The antiallergic drug cetirizine showed significant *in vitro* antimicrobial activity against 51 strains of bacteria belonging to 5 Gram-positive and 4 Gram-negative genera. The minimum inhibitory concentration of the drug determined both by agar dilution and broth dilution method ranged from 200-2000 µg/ml against most of the bacteria tested. Cetirizine was bactericidal in action *in vitro* on both Gram-positive and Gram-negative bacteria. *In vivo* experiment with this drug proved that it could offer significant protection to mice challenged with a virulent bacterium *Salmonella typhimurium* NCTC74. Thus, cetirizine has immense potential to be developed as an antibacterial agent. Therefore the present study would help in corroborating future prospects in disseminating efficacious use of cetirizine in mitigation of microbial infections.

Key words: Non-antibiotic, antiallergic, antibacterial, bactericidal

The discovery of antimicrobial drugs, often a matter of chance and serendipity till the end of the 19th century, gradually become an exercise of detailed scientific knowledge and wisdom, which resulted in “drug explosion”. Among the antimicrobial agents, largest share belongs to the antibiotics. Enormous use of antibiotics has led not only to emergence of drug resistant bacteria, but also to increasing infections with opportunistic microorganisms. Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Their advent changed the outlook of the physician about the power of drugs on diseases. As a class, they are one of the most frequently used as well as misused drugs. So, pharmaceutical industries and research organizations are constantly making an effort to synthesize new antibiotics to combat drug resistance. Extensive studies of various workers detect antimicrobial action in different types of drugs belonging to different pharmacological classes, such as antihistamines like bromodiphenhydramine and diphenhydramine^[1], methdilazine^[2],

promethazine^[3], trimeprazine^[4], terfenadine^[5], tranquilizers like promazine^[6], antihypertensives like propranolol^[7], methyl dihydroxyphenylalanine (methyl DOPA)^[8], dobutamine^[9], amlodipine^[10], oxyfedrine^[11], lacidipine^[12], antispasmodics like dicyclomine^[13,14], antipsychotics like chlorpromazine^[15], fluphenazine^[16], thioridazine^[17], prochlorperazine^[18], flupenthixol^[19], antiinflammatory agents like diclofenac^[20-24], flurbiprofen^[25] and sympathomimetic drug dopamine hydrochloride^[26]. Such drugs, having antimicrobial activity in addition to their predesignated pharmacological activity, have been grouped together under the banner of “non-antibiotics”^[27].

The present study was performed with cetirizine, an antiallergic drug, to observe its antimicrobial activity

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by *in vitro* and *in vivo* experiments. The antimicrobial activity of this non-antibiotic could be useful in the fight against antimicrobial resistance.

MATERIALS AND METHODS

Liquid media used in this study were peptone water (PW) containing 1.0% peptone (Oxoid) plus 0.5% AnalaR NaCl, nutrient broth (NB, Oxoid) and Muller Hinton Broth (MHB, Oxoid). Solid media were nutrient agar (NA), prepared by solidifying NB with 2.0% agar (NA, Oxoid), and Muller-Hinton agar (MHA, Oxoid), pH 7.2-7.4.

Total 51 bacterial strains belonging to 5 Gram-positive and 4 Gram-negative genera, comprising of 13 Gram-positive and 38 Gram-negative strains were tested (Table 1, fig. 1). Many of the strains were of human

origin, identified^[28-30] and preserved in freeze dried state. Many of the standard strains like *Staphylococcus aureus* (ATCC 29157), *Bacillus subtilis* (ATCC 6633), *Micrococcus lutea* (ATCC 9341), *Vibrio cholerae* (ATCC 14033), *Escherichia coli* (ATCC 25922) and even multidrug resistant (MDR) strains like *E. coli* (R239), *E. coli* (R 224), *Vibrio cholerae* (DN 8), *S. aureus* (ML 145) and *S. aureus* (ML 335) were included in the study. All microorganisms were maintained at 4° at slant culture for a maximum of one month and as freeze dried culture for long term preservation^[31].

Preparation of cetirizine stock solution:

Cetirizine used in this study was obtained as pure dry powder of pharmaceutical grade. Specified amount of the drug was accurately weighed and transferred into a

TABLE 1: PRELIMINARY SCREENING OF CETIRIZINE AS AN ANTIMICROBIAL AGENT BY AGAR DILUTION METHOD

Name of bacteria	Concentration of cetirizine (µg/ml)					
	0 (control)	200	400	1000	1400	2000
<i>Staphylococcus aureus</i> ML281	+	+	+	-	-	-
<i>Streptococcus faecalis</i> S ₁	+	+	+	-	-	-
<i>Bacillus subtilis</i> ATCC6633	+	+	+	-	-	-
<i>Micrococcus lutea</i> ATCC9341	+	+	-	-	-	-
<i>Salmonella typhi</i> D1716	+	-	-	-	-	-
<i>Salmonella typhi</i> D642	+	+	+	+	-	-
<i>Salmonella typhi</i> D1604	+	+	+	+	-	-
<i>Vibrio cholerae</i> ATCC14033	+	+	+	-	-	-
<i>Shigella dysenteriae</i> NCTC566/61	+	+	+	-	-	-
<i>Escherichia coli</i> 306	+	+	+	-	-	-
<i>Escherichia coli</i> ATCC25922	+	+	+	+	+	-

+ Presence of growth; - absence of growth

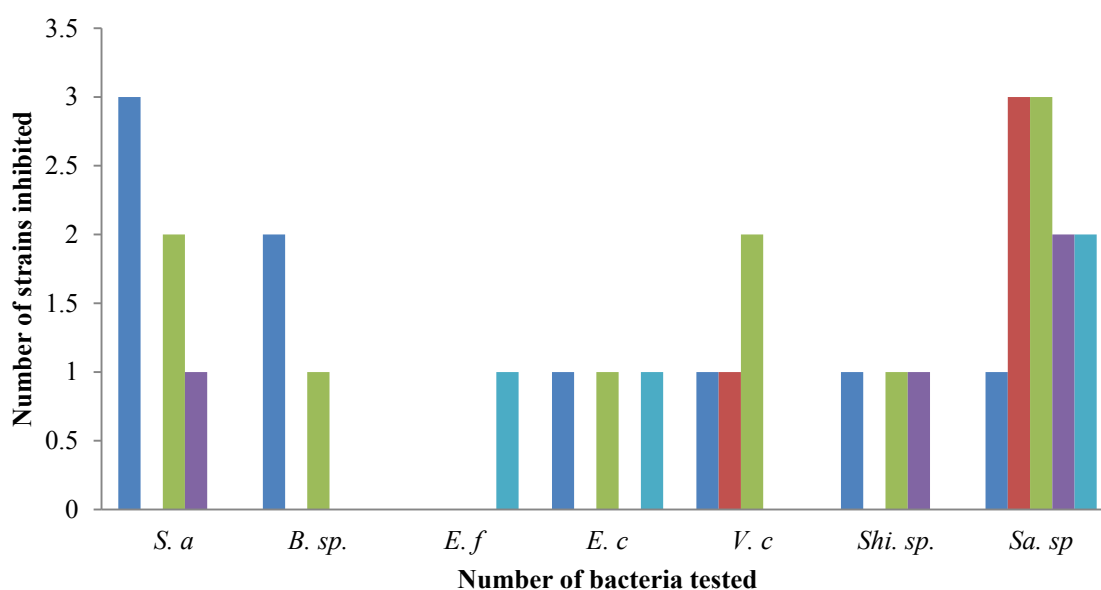


Fig. 1: Antibacterial spectrum of cetirizine

■ 200 µg/ml, ■ 400 µg/ml, ■ 1000 µg/ml, ■ 1400 µg/ml, ■ 2000 µg/ml. *S. a*: *Staphylococcus aureus*; *B. sp.*: *Bacillus sp.*; *E. f*: *Enterococcus faecalis*; *E. c*: *Escherichia coli*; *V. c*: *Vibrio cholerae*; *Shi. sp.*: *Shigella sp.*; *Sa. sp.*: *Salmonella sp.*

suitable sterile volumetric flask and dissolved in sterile distilled water. The flask is covered properly to protect it from light.

Determination of minimum inhibitory concentration (MIC) of cetirizine:

MIC of cetirizine was accurately determined with respect to different test bacteria following the standard guideline of agar dilution techniques as described by the clinical laboratories and standard institutes (CLSI 2006). For this purpose, cetirizine was dissolved in sterile distilled water and added to molten Muller Hinton Agar (MHA) at concentration of 0 (control), 200, 400, 1000, 1400 and 2000 µg/ml. Gram-positive bacteria were grown in NB and Gram-negative bacteria in PW for 18 h and bacteria were harvested during the secondary growth phase. A suspension of organism was prepared in 5 ml sterile distilled water. The turbidity of the suspension was adjusted to a 0.5 McFarland standard with a UV/Vis spectrophotometer (Chemito UV 2600 double beam spectrophotometer) at 625 nm, which corresponded to 2.4×10^8 CFU/ml. The inocula were prepared by further diluting the suspension 1:100 with sterile distilled water in such a manner that a 2 mm (internal diameter) loopful of culture contain 10^5 CFU. These were spot inoculated on the MHA plates containing increasing concentrations of the drug including the control. The plates were incubated at 37° and examined for the appearance of growth after 24 h (extended up to 72 h, if necessary). The MIC was determined as the concentration of the drug that resulted in no visible growth.

Determination of effect of cetirizine on *S. aureus* ML 281 and *Salmonella typhi* 62:

To determine the bacteriostatic or bactericidal action of the antihistaminic compound, strains that are sensitive to cetirizine was selected and each of them was grown in 4 ml NB for 18 h. From that 18 h old broth culture, 2 ml was taken and added into another 4 ml of fresh NB. This was incubated at 37° for 2 h so that the bacterial culture could attain logarithmic growth phase. The number of viable cells in the culture was determined by the CFU count technique as described by Miles and Mishra (1938)^[34]. At this stage cetirizine was added at a concentration higher than the respective MIC values against the selected sensitive stains. CFU counts from the culture were individually taken after 2 h, 6h and finally after 18 h^[32].

***In vivo* antimicrobial activity:**

Male albino mice of Swiss strain weighing 18-20 g

were taken for *in vivo* study. Animals were maintained at standard conditions at $21 \pm 1^\circ$ and 50-60% relative humidity with a 14 h photo period. Water and dry pellet diet were given *ad libitum*. The virulence of the test strain *S. typhimurium* NCTC74 was exalted by repeated mouse passages and the median lethal dose (MLD or LD₅₀) of the passaged strains was determined. From this the 50×MLD of the strains corresponding to 0.95×10^9 CFU/mouse suspended in 0.5 ml NB served as the challenge dose^[33] for all groups of animals. Reproducibility of the challenged dose was ensured by standardization of its optical density in a colorimeter at 640 nm and determination of the CFU count in NA^[34].

To determine the toxicity of cetirizine, 40 mice were taken, 20 of which were injected with 60 µg of drug while the remaining 20 received 100 µg of cetirizine. They were kept under observation for up to 100 h. The protective capacity of cetirizine was judged as follows: two groups of mice, 20 animals per group were kept in separate cages. Group I was intraperitoneally administered 60 µg cetirizine per mouse and group II was given 100 µg of the drug per mouse. After 3 h each group was challenged with 50 MLD of *S. typhimurium* NCTC 74. A control group of 40 mice was also injected similarly with the same bacterial strain and 0.1 ml sterile saline instead of cetirizine. The protective capacity of the drug was determined by recording the mortality of mice in different groups up to 100 h of treatment and statistically by chi square test.

In another study, 4 groups of 5 mice were used. Group 1 and 3 were injected 100 µg of cetirizine intraperitoneally and each mice of group of 2 and 4 received 0.5 ml of sterile saline instead of drug. After 3 h of treatment, all groups were given 50 MLD challenges of *S. typhimurium* NCTC74. After 2 h, all mice of group 1 and 2 were sacrificed and their heart blood was collected, livers and spleens were separated aseptically and homogenized in tissue homogenizers. CFU count of individual organs was determined separately. The same procedure was applied to groups 3 and 4, 18 h after challenge. The data obtained were statistically analyzed by student t-test. All the animal experiments were carried out following Institutional Animal Ethical Committee guidelines (955/A/06/CPSEA2006).

RESULTS AND DISCUSSION

A primary screening of cetirizine against 7 bacteria belonging to Gram-negative and 4 Gram-positive genera shows satisfactory antimicrobial activity against most of the test bacteria (Table 1). In an

elaborate *in vitro* study, the drug was tested against 42 different strains of bacteria belonging to both Gram-positive and Gram-negative genera. Six *S. aureus* three *Bacillus* sp., one *Enterococcus*, Three *E. coli*, four *V. cholera*, three *Shigells* and twenty two *Salmonella* sp. were used in the elaborate study. Many of them were inhibited at 200-2000 µg/ml concentration; few were also susceptible below 200 µg/ml concentration. The order of sensitivity towards cetirizine was *Bacillus* sp., *Vibrio cholera*, *S. aureus*, *Escherichia coli*, *Shigella* sp. But few strains of *S. aureus*, *E. coli*, *Shigella* sp. and *Salmonella* sp. were not inhibited at test concentration.

The MIC of cetirizine against *S. typhi* 62 and *S. aureus* ML281 was 1000 µg/ml; in logarithmic growth phase their CFU count was 5×10^6 CFU/ml for both of them. At 0 h, $2 \times \text{MIC}$ of cetirizine of the test organisms was added to each of the culture tubes. Subsequently, when the CFU counts were determined after 2, 6, and 18 h, it was noticed that there was a gradual decrease in the number of viable cells up to 6 h for both bacteria. The decrease in CFU count were 5×10^{10} , 4×10^{10} , 2×10^7 in the case of *S. aureus* ML281 whereas 5×10^{10} , 1×10^8 , 1×10^6 in the case of *S. typhi* 62, respectively. However there were no viable cells found after 18 h, proving the bactericidal property of drug (fig. 2).

Table 2 shows that in control group 49 out of 60

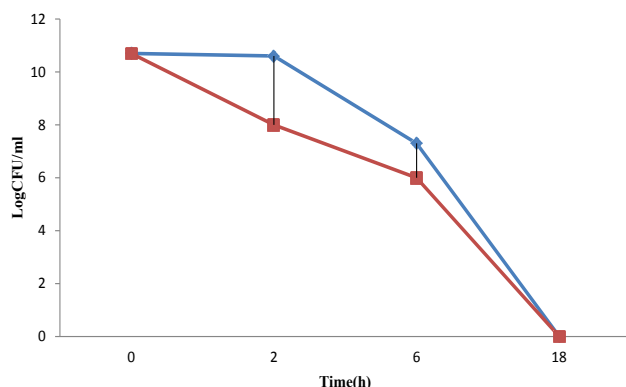


Fig. 2: The mode of action of cetirizine on *S. aureus* ML281 and *S. typhi* 62

◆ *S. aureus* ML281, ■ *S. typhi* 62

TABLE 2: DETERMINATION OF *IN VIVO* PROTECTION BY CETIRIZINE

Control group*		Test group*	
Drug injected per mouse(µg)	Mice died out of 60	Drug injected per mouse (µg)	Mice died out of 20
0.5 ml sterile saline	49	30	13
		60	4
		100	0

*Received challenged dose of 0.95×10^9 CFU in 0.5 ml NB of *S. typhimurium* NCTC74

animals died within 100 h of challenge. No mortality was recorded in those groups that received highest concentration of drug (100 µg/ml). As can be seen in Table 3, by comparing the CFU count in heart blood, liver and spleen at 2 h and 18 h, it is evident that there is no significant increase in viable count in drug treated group even after 18 h, thus clearly indicating the bacteriostatic nature of cetirizine (fig. 3).

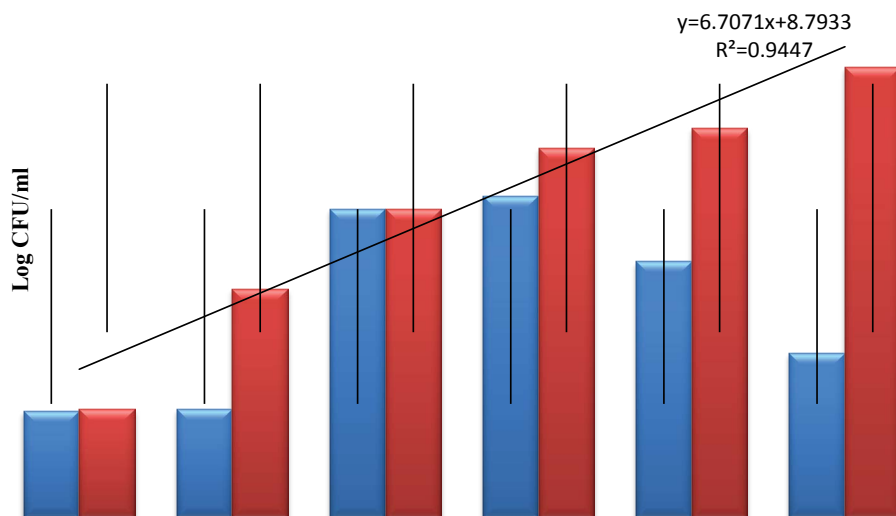
The search for antimicrobials has now been extended to a class of compounds named non-antibiotics which are employed for the therapy of noninfectious pathologies and which demonstrate significant antimicrobial activity against some of the most pathogenic infectious agents such as vancomycin resistant or methicillin resistant *S. aureus*^[35] or MDR *Mycobacterium tuberculosis*^[36-38].

Cetirizine dihydrochloride is an antagonist of histamine, mostly against H_1 receptor. It inhibits effect of histamine in H_1 receptor of smooth muscle. It also blocks capillary permeability to prevent edema, but as a second generation ethanolamine, it does not cause sedation. Cetirizine HCl is a white powder having a molecular weight of 461.8. Chemically it is (\pm)-2-[2-[4-[(4-chlorophenyl)phenylmethyl]ethoxy]acetic acid dihydrochloride (fig. 4). This drug is sensitive to light and freely soluble in water, partially insoluble in acetone and methyl chloride^[39,40].

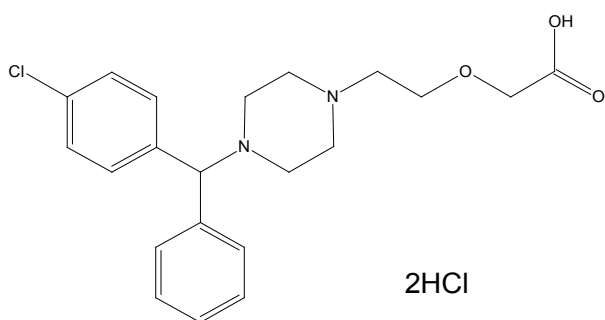
Cetirizine being a H_1 receptor antagonist is used in conditions like upper respiratory allergies, pollinosis, urticarial/atopic dermatitis; also used as adjuvant in seasonal asthma. It is a metabolite of hydroxyzine with marked affinity for peripheral H_1 receptor; penetrates brain poorly but subjective drowsiness has been experienced at higher doses. It is not metabolized, does not prolong cardiac action potential or produce arrhythmias when given with erythromycin, ketoconazole. Cetirizine also inhibits release of histamine and cytotoxic mediators from platelets as well as eosinophil chemotaxis during the secondary phase of allergic response. Thus it may benefit allergic disorder by other actions as well. It attains high and longer lasting concentration in skin which may be responsible for superior efficacy in urticarial/atopic dermatitis, as well as once daily dosing although the elimination half-life ($t_{1/2}$) is 7-10 h^[41,42]. In addition to these pharmacological actions, cetirizine has significant activity on several Gram-positive and Gram-negative bacteria *in vitro* and *S. typhimurium in vivo*. The study reaffirms the antimicrobial activity of this class of drugs.

TABLE 3: EFFICACY OF CETIRIZINE IN REDUCING BACTERIAL COUNTS IN CHALLENGED MICE

Time of sampling (h)	Group	No. of mice	Drug conc. per mouse	CFU/ml counts in		
				Heart blood	Liver	Spleen
2	I	5	Cetirizine (1 mg)	2×10^2 , 2×10^2 , 1×10^2 , 2×10^2 , 2×10^2	5×10^6 , 1×10^6 , 2×10^6 , 3×10^6 , 5×10^6	2.5×10^5 , 2×10^5 , 3.5×10^5 , 1.5×10^5 , 2×10^5
2	II	5	Saline (control)	2×10^2 , 3×10^2 , 1×10^2 , 2×10^2 , 2×10^2	5×10^6 , 1×10^6 , 2×10^6 , 3×10^6 , 5×10^6	1.5×10^8 , 1×10^8 , 1.5×10^8 , 1×10^8 , 2×10^8
18	III	5	Cetirizine (1 mg)	1.5×10^2 , 2×10^2 , 2×10^2 , 2.5×10^2 , 1.5×10^2	5×10^6 , 5×10^6 , 5×10^6 , 4×10^6 , 7×10^6	5×10^3 , 2×10^3 , 3×10^3 , 5×10^3 , 1×10^3
18	IV	5	Saline (control)	5×10^4 , 6×10^5 , 4×10^4 , 6×10^4 , 1×10^4	4×10^7 , 5×10^7 , 5×10^7 , 6×10^7 , 6×10^7	4×10^9 , 5×10^8 , 4×10^9 , 3×10^9 , 4×10^9

**Fig. 3: Efficacy of cetirizine at different organs in different time**

■ log CFU/ml counts in cetirizine HCl (1 mg), ■ log CFU/ml counts in saline (control)

**Fig. 4: Structure of cetirizine dihydrochloride**

Most of the bacteria tested were inhibited within 1000 $\mu\text{g/ml}$ concentration of the drug whereas few Gram-positive and Gram-negative bacteria were killed by the drug at much lower concentration (200-400 $\mu\text{g/ml}$) of the drug. In an *in vitro* study, cetirizine is proved as a bactericidal agent, which was performed in a Gram-positive bacteria *S. aureus* ML281 and a Gram-negative bacteria *S. typhi* 62. So from the *in vitro* study it can be concluded that cetirizine produced antimicrobial activity at around 1000 $\mu\text{g/ml}$ concentration but in the *in vivo* study cetirizine

gave significant protection to the challenged mice (with *S. typhimurium* NCTC74) at 100 $\mu\text{g/ml}$ concentration. In another *in vivo* study where the viable count of organ homogenates and heart blood were compared to control and drug treated challenged mice, the result were highly significant ($P < 0.5$). Examinations among various classes of pharmacological agents have revealed that in general the tricyclic phenothiazines possess discernible antimicrobial action^[43]. Extensive reviews of literature have revealed that antimicrobial properties of several phenothiazines and other antimicrobial agents are due to the presence of aromatic rings^[44-46].

Antihistaminic drug cetirizine contains aromatic ring and piperazine ring with halogens. The promising antimicrobial activity of this drug may be attributed to these structural components. Thus, cetirizine stands a chance of being developed as an antimicrobial agent to combat microbial resistance and bacterial infection associated with allergic reactions.

The main limiting factor of non-antibiotics to display

their antimicrobial characteristics in mammalian system is that the maximum serum level remains (approximately 1 mg per liter) lower than the concentration required for inhibiting microbial growth. However this level might be sufficient to modify microbial metabolism and act synergistically with certain antibiotics^[47]. On the other hands the currently published information describes *in vitro* and *in vivo* efficacy in animals. There is very limited clinical information that indicates clinically relevant activity of non-antibiotics in human. In addition, there is a need to take thermodynamics into account *in vivo*. On the basis of this information new approaches to the infection can be easily designed.

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Conflicts of interest:

There are no conflicts of interest.

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