Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 6 pp. 1269–1278, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACOLOGY

CITALOPRAM AND VENLAFAXINE DIFFERENTIALLY AUGMENTS ANTIMICROBIAL PROPERTIES OF ANTIBIOTICS

MOHAMMAD AYAZ[1]*, FAZAL SUBHAN[2], JAWAD AHMED[3], ARIF-ULLAH KHAN[4], FARHAT ULLAH[1], ABDUL SADIQ[1], NAWAZISH-I-HUSAIN SYED[5], IHSAN ULLAH[6] and SAJID HUSSAIN[7]

[1]Department of Pharmacy, University of Malakand, Dir (L), Pakistan
[2]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[3]Institute of Basic Medical Sciences, Khyber Medical University (KMU) Peshawar, Pakistan
[4]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan
[5]University College of Pharmacy, University of The Punjab Lahore, Lahore-54000, Pakistan
[6]Department of Pharmacy, University of Swabi, Swabi, Pakistan
[7]Department of Pharmacy, Kohat University of Science and Technology, Kohat, Pakistan

Abstract: We investigated intrinsic antibacterial potential of citalopram (CIT) and venlafaxine (VF) alone and in combination with seven antibiotics against clinical isolates of Gram positive and Gram negative bacteria, ATCC strains and to evaluate their effect on reversal of antibiotic resistance. Intrinsic antibacterial action of CIT, VF and MICs were determined using well assay, nutrient broth and agar dilution techniques. Disk diffusion method was used to study bacterial susceptibility to CIT and VF. Intrinsic antibacterial assay revealed that CIT possesses mild to moderate intrinsic bactericidal properties, while VF was found inactive. CIT and VF augmented the antibacterial effects of antibiotics and some previously resistant strains were converted to susceptible range. Antibacterial activities of levofloxacin, moxifloxacin and gentamicin were significantly increased against *S. aureus* in combination with 310 µg/mL of CIT. Moxifloxacin, levofloxacin and gentamicin activity against *E. coli* was significantly increased with the addition of 600 and 1200 µg/mL of VF, respectively ($p < 0.05$). Resistance of *E. coli* to cefixime and *P. aeruginosa* to cloxacillin were reversed with addition of increasing concentration of CIT. Further, resistance of *S. aureus* ATCC 6538 to cefixime, *E. coli* to cefixime, cloxacillin and *P. aeruginosa* resistance to cloxacillin were reversed with increasing concentrations of VF. CIT and VF also showed activity against clinical isolates of *Salmonella typhi, E. coli, Klebsiella pneumoniae, Proteus mirabilis, S. aureus, P. aeruginosa* and *Enterococcus faecalis*, exhibiting minimum inhibitory concentrations from 6 to 7 mg/mL. Combination study revealed that these drugs are resistance modifying agents in combination with antibiotics.

Keywords: intrinsic antibacterial assay, ATCC, MDR, citalopram, venlafaxine

Antimicrobial drug resistance is a major challenge in the chemotherapy of infectious diseases and cancer (1, 2). Factors leading to multidrug resistance (MDR) include, reckless utilization, sustained over reliance on antimicrobial agents, target site modification and active drug efflux mediated by efflux pumps (3, 4). To overcome MDR, antibiotics are administered along with inhibitor drugs, which will restore the activity of antibiotics by inhibition of inactivating enzymes (5) or increase the availability of antibiotics at the target site by reducing their extrusion out of the target site (6, 7).

Different compounds used for the management of non-infectious pathological conditions known to exhibit antimicrobial activity against a variety of microbes are known non-antibiotics (8). Among the non-antibiotic compounds, several have been reported to synergistically interact and augment the activity of antibiotics against a variety of microorganisms (8, 10). In the recent years, it is reported that selective serotonin reuptake inhibitors (SSRIs) demonstrate antibacterial characteristics (11) and can reverse *Plasmodium* resistance to antimalarial drugs (12). Selective serotonin reuptake inhibitors are

* Corresponding author: e-mail: ayazuop@gmail.com; phone: +92-346-8004990

mainly effective against Gram positive microbes, yet are less effective against the majority of Gram negative bacteria (11). Sertraline - an SSRI, has been reported to possess antifungal activity against *Candida* species (13). The same group of investigators reported that a group of SSRI (fluoxetine, sertraline, paroxetine, citalopram) and nor-epinephrine reuptake inhibitor (NRI) reboxetine, inhibit the growth of *Aspergillus* species *in vitro* (14).

Phenothiazines and antidepressants have been reported to reverse multidrug resistant phenotypes of bacteria and make them susceptible to previously resistant antibiotics (15, 17). Paroxetine and femoxetine are known to potentiate antimicrobial activity of several antibiotics against Gram positive bacteria (18). Kaatz et al. investigated the activity of these compounds and their derivatives against *S. aureus* and *E. coli* (19). The assayed compounds augmented the effect of various antimicrobial agents against multidrug resistance efflux pumps including NorA, non NorA, AcrAB-TolC pumps and inhibited the activity of RND efflux pumps (20). Paroxetine in a concentration of 25 µg/mL afforded eight fold decrease in minimum inhibitory concentration (MIC) of norfloxacin against *S. aureus* 1199B a NorA producing strain and k2068 a non-NorA phenotype (19). Antimicrobial activities of three phenothiazines: promazine, thioridazine, triflupromazine and two tricyclic antidepressants: amitriptyline and imipramine were investigated by Hendricks et al., against *S. aureus*, *P. aeruginosa* and *Klebsiella pneumonia* (21). The phenothiazine derivatives thioridazine and triflupromazine showed notable activity against *S. aureus* and *P. aeruginosa* strains. Evidence also indicates that SSRIs inhibit the expression of P-glycoprotein's, primary active transporters responsible for the extrusion of several chemotherapeutic agents from their site of action (22). Based on these investigations, some newly developed antidepressants including CIT and VF were selected for further antibacterial evaluation alone and in combination with antibiotics.

EXPERIMENTAL

Chemicals and drugs

Citalopram (CIT) and venlafaxine (VF) were kindly provided by Universal Pharmaceuticals and Usawa Pharmaceuticals, Pakistan, respectively. Antibiotic powder of ciprofloxacin, levofloxacin, norfloxacin, moxifloxacin, cefixime, cloxacillin, and gentamicin were purchased from Sigma Aldrich, USA. Antibiotic discs (Oxoid) and dimethyl sulfoxide (DMSO) Labscan Patumwan, Bankok 10330, Thialand, were used in the study.

Bacterial strains

Control strains including *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739) and *P. aeruginosa* (ATCC 9027) were kindly provided by CIRIN Pharmaceuticals (Pvt.) Ltd., Pakistan. Clinical isolates were collected from Microbiology Department of Khyber Teaching Hospital (KTH) Peshawar, Pakistan and were identified by different biochemical tests (23). Bacteria were preserved in freeze-dried condition at 4^{o}C in stab slant agar until later use.

Culture media

Nutrient agar (Oxoid Ltd., UK), mannitol salt agar (Oxoid Ltd., England, CM0085), MacKonkeys agar (Oxoid Ltd., England, CM0115), triple sugar iron (TSI) agar (Oxoid Ltd., England, CM0277), CLED medium (Oxoid Ltd., England, CM0423), DNASE agar (Oxoid Ltd., England, CM0321), Simmons citrate agar (Oxoid Ltd., England, CM0155), urea agar base (Oxoid Ltd., England, CM0053), nutrient broth base powder (Oxoid) were used for the growth and identification of the selected microorganisms according to the guidelines of Clinical Laboratory Standards Institute (CLSI) and manufacturer specifications.

Preparation of stock solutions

Depending on solubility, CIT was dissolved in dimethyl sulfoxide (DMSO) and serial twofold dilutions were made, ranging from 310 to 5000 µg/mL under laminar flow hood. VF solutions were prepared in distilled water ranging from 75 to 1200 µg/mL. Sterilization of the stock solutions were performed by passing it through syringe filters of Minisart (Sartorious) 0.2 µm size in safety cabinets.

Standardization of bacterial suspension

Bacterial cultures were grown for 24 h at 37^{o}C and suspensions with cell density of 10^{8} CFU/mL, were prepared using McFarland standard and further diluted to a cell density of 10^{6} CFU/mL using a UV visible spectrophotometer (Thermo Electron Corporation, USA) at 625 nm and the standardization was maintained for the period of the study.

Intrinsic antibacterial studies on citalopram and venlafaxine

Intrinsic antibacterial action of CIT and VF were determined using agar dilution and well assay methods. In agar dilution method, CIT and VF solutions corresponding to 310-5000 µg/mL and 75-1200 µg/mL, respectively, were aseptically added to sterile molten MHA at 40^{o}C (24). One loopful of the

already prepared bacterial suspension was inoculated on the MHA plates containing increasing concentration of CIT and VF. Plates were incubated at 37°C for 24 h and were examined for the appearance of growth. In well assay method, bacterial plates were inoculated by swabbing MHA plates with already prepared bacterial suspensions under laminar flow hood (25, 27). Wells of 6 mm diameter were bored into the MHA plates using sterilized cork borer. After drying the bores were filled with 100 µL of CIT, VF and antibiotics solutions taking care not to let spillage of the solutions on the surface of the agar. The plates were incubated at 37°C for 24 h. Zone of inhibition were measured around the bores.

Determination of minimum inhibitory concentrations (MICs)

For determination of MICs both nutrient broth and agar dilution methods approved by CLSI were used (28, 29). For these tests, CIT in concentration of 310-5000 µg/mL and VF 75-1200 µg/mL were added to sterilized tube containing nutrient broth and were inoculated with the test microbes. Tubes were incubated using shaker incubator at 37°C for 24 h. MIC was considered that concentration at which no visible bacterial growth was observed. All experiments were done in five replicates.

***In vitro* synergy between citalopram, venlafaxine and antibiotics**

The combined antibacterial effect of CIT, VF and antibiotics were determined by disk diffusion method as described by CLSI (30). Sterile filter paper disks (Whatman no. 1, 7.25 mm diameter) were prepared using method described previously (28). In combination study, solutions corresponding to 5 µg of ciprofloxacin, levofloxacin, moxifloxacin, cefexime, cloxacillin, 10 µg of norfloxacin and gentamicin, 310-500 µg of CIT and 75-1200 µg of VF were added to these disks. Antibiotic disks of these drugs available were used as positive control.

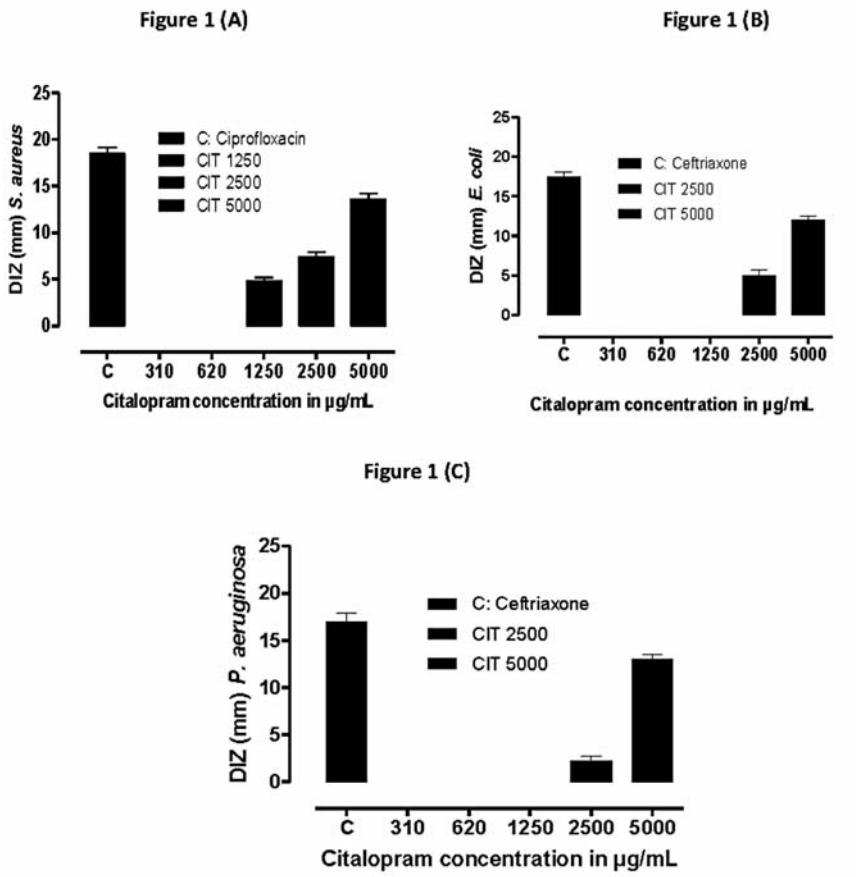

Figure 1. Intrinsic antibacterial activities of citalopram against *Staphylococcus aureus* (A), *Escherichia coli* (B) and *Pseudomonas aeruginosa* (C)

Table 1. Diameter of inhibitory zones of selected antibiotics with increasing concentrations of citalopram (310-5000 µg/mL) against *S. aureus* ATCC 6538.

Antibiotic	Diameter of the inhibitory zone (mm); the mean ± SEM (n = 5)					
	Control	Antibiotic + CIT 310 µg/mL	Antibiotic + CIT 620 µg/mL	Antibiotic + CIT 1250 µg/mL	Antibiotic + CIT 2500 µg/mL	Antibiotic + CIT 5000 µg/mL
Ciprofloxacin 5 µg	27.50 ± 0.57	29 ± 0.81	30.75 ± 0.50*	34.25 ± 0.95***	39 ± 0.00***	43 ± 2.30***
Levofloxacin 5 µg	24.25 ± 0.50	27.5 ± 1.00***	30 ± 0.0***	32.5 ± 0.57***	37.5 ± 0.57***	40 ± 0.00***
Norfloxacin 10 µg	24.25 ± 0.50	25.75 ± 0.50	28.5± 0.57***	31.25 ± 0.5***	37.5 ± 1.73***	40 ± 0.00***
Moxifloxacin 5 µg	29 ± 0.00	31.5 ± 0.57**	34.5 ± 1.0***	36.5 ± 1.29***	38.5 ± 0.57***	40.5 ± 0.57***
Cefixime 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Cloxacillin 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Gentamycin 10 µg	28.75 ± 0.95	30.50 ± 0.57**	35 ± 0.00***	39.25 ± 0.95***	40.5 ± 0.57***	42.5 ± 0.57***

*: Values significantly different as compared to antibiotic treatment group only, *p < 0.05, ** p < 0.01, *** p < 0.001. Control: antibiotic only. CIT: citalopram.

Table 2. Diameter of inhibitory zones of selected antibiotics with increasing concentrations of venlafaxine (VF 75-1200 µg/mL) against *S. aureus* ATCC 6538.

Antibiotic	Diameter of the inhibitory zone (mm); the mean ± SEM (n = 5)					
	Control	Antibiotic + VF 75 µg/mL	Antibiotic + VF 150 g/mL	Antibiotic + VF 300 µg/mL	Antibiotic + VF 600 µg/mL	Antibiotic + VF 1200 g/mL
Ciprofloxacin 5 µg	28.6 ± 3.13	32.6 ± 3.97	33.6 ± 3.97	34 ± 3.74	35.8 ± 3.19*	39.8 ± 1.09***
Levofloxacin 5 µg	27.8 ± 1.09	32.2 ± 3.27	33.8 ± 3.19	34 ± 3.39*	35.6 ± 3.36**	38.8 ± 1.64***
Norfloxacin10 µg	25.4 ± 0.89	29.8 ± 3.19	32 ± 3.93*	34 ± 3.46**	34.6 ± 3.97**	40.2 ± 4.14***
Moxifloxacin 5 µg	28.6 ± 2.07	33.6 ± 2.07**	34.4 ± 1.94**	35.4 ± 1.140***	37.2 ± 2.16***	39.6 ± 3.20***
Cefixime 5 µg	0 ± 0	5.2 ± 4.816	5.4 ± 5.07	7 ± 4.12*	10.4 ± 0.54***	12.6 ± 1.34***
Cloxacillin 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Gentamycin 10 µg	29.6 ± 0.54	33 ± 1.22	34.6 ± 1.67*	35.2 ± 1.30***	35.4 ± 1.34***	38.2 ± 3.42***

*: Values significantly different as compared to antibiotic treatment group only, *p < 0.05, ** p < 0.01, *** p < 0.001. Control: antibiotic only. VF: venlafaxine.

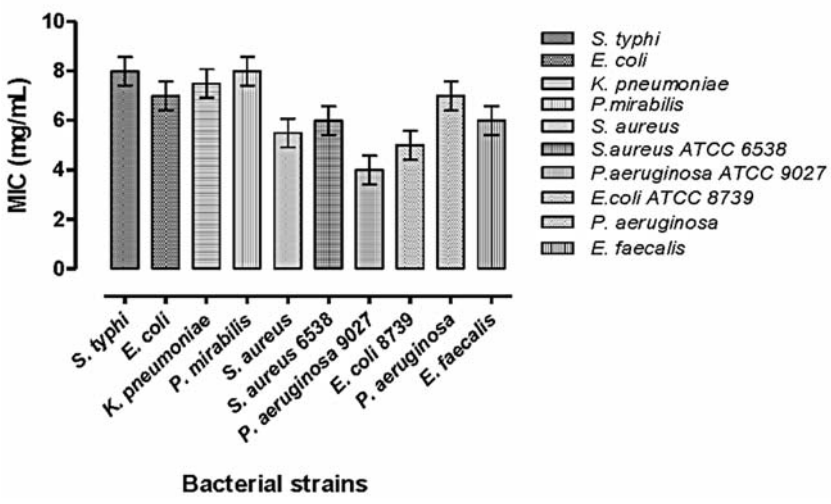

Figure 2. Minimum inhibitory concentrations (MICs) of citalopram against bacterial strains

Overnight grown bacterial culture was used to prepare bacterial suspensions. From these suspensions 100 µL were uniformly spread over the surface of already prepared MHA plates under laminar flow hood and were allowed to dry. Disks containing antibiotic alone and increasing concentrations of CIT and VF were placed equidistantly on the surface of inoculated plated and were incubated at 37°C for 24 h. Diameter of inhibitory zones of antibiotic alone and in combination with CIT and VF were determined according to CLSI standards for zone interpretation (31).

Statistical analysis

One-way ANOVA followed by Dunnett's multiple comparison test were applied for the comparison of positive control with the test groups. All the experiments were repeated in five replicates and values were expressed as the means ± S.E.M. The p values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Intrinsic antibacterial activity and MICs of citalopram and venlafaxine

In intrinsic antibacterial study, CIT exhibited moderate activity, whereas VF was completely devoid of intrinsic activity even at highest concentration used (Fig. 1 A,B). Intrinsic activity of CIT was more prominent against Gram-positive bacteria i.e., *S. aureus* (Fig. 1A, 2) and inhibitory zones were increased along the concentration of CIT scoring inhibitory zone of 5, 8 and 14 mm at concentrations of 1250, 2500 and 5000 µg/mL, respectively. CIT at concentrations of 2500 and 5000 µg/mL produced inhibitory zones of 5 and 12 mm, respectively, against *E. coli* while against *P. aeruginosa* CIT was only effective at higher concentration (5000 µg/mL) scoring inhibitory zone of 13 mm. MICs of CIT against of *S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, were 4, 5, and 4 mg/mL, respectively, whereas MICs for clinical isolates were: *Salmonella typhi* 6 mg/mL, *E. coli* 7 mg/mL, *K. pneumonia* 6 mg/mL, *P. mirabilis* 6 mg/mL, *S. aureus* 5 mg/mL, *P. aeruginosa* 6 mg/mL and 7 mg/mL for *Enterococcus faecalis* 29212 (Fig. 2). MICs of VF against these bacteria were too high to be clinically important and were above concentrations that were used in combination with antibiotics.

Effect of increasing concentrations of citalopram and venlafaxine on the susceptibility of *S. aureus*

The activity of antibiotics were significantly improved with the addition of increasing concentrations of CIT and VF (Tables 1, 2). The combined anti-staphylococcal activity was more pronounced for levofloxacin, moxifloxacin and gentamicin, whereby only 310 µg/mL of CIT has significantly increased the diameter of inhibitory zones $p \leq 0.001$, ≤ 0.01 and ≤ 0.01, respectively, in comparison to antibiotic treated groups only. *S. aureus* was found to be resistant to cefixime and cloxacillin and this resistance was not reversed even at the highest concentration being used. VF was hypothesized to be

Table 3. Diameter of inhibitory zones for selected antibiotics with increasing concentrations of citalopram (CIT 310-5000 µg/mL) against *E. coli* ATCC 8739.

Antibiotic	Diameter of the inhibitory zone (mm); the mean ± SEM (n = 5)					
	Control	Antibiotic + CIT 310 µg/mL	Antibiotic + CIT 620 µg/mL	Antibiotic + CIT 1250 µg/mL	Antibiotic + CIT 2500 µg/mL	Antibiotic + CIT 5000 µg/mL
Ciprofloxacin 5 µg	26.0 ± 1.41	26.5 ± 0.70	28.5 ± 0.70	30.0 ± 1.41*	30.5 ± 0.70*	36.5 ± 2.12***
Levofloxacin 5 µg	25.5 ± 0.70	26.5 ± 0.70	27.5 ± 0.70	29.5 ± 0.70**	31.0 ± 1.41**	34.5 ± 0.70***
Norfloxacin 10 µg	24.0 ± 0.00	24.0 ± 0.00	25.5 ± 0.70	27.5 ± 0.70**	30.0 ± 0.00***	32.0 ± 0.70***
Moxifloxacin 5µg	26.5 ± 0.70	26.5 ± 0.70	28.0 ± 0.00	29.5 ± 0.70*	30.5 ± 0.70**	34.0 ± 0.70***
Cefixime 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	10.0 ± 0.00
Cloxacillin 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Gentamycin 10 µg	23.0 ± 2.82	26.0 ± 1.41	28.0 ± 1.41*	30.0 ± 0.00*	31.5 ± 0.70**	35.5 ± 0.70***

*: Values significantly different as compared to antibiotic treatment group only, *p < 0.05, ** p < 0.01, *** p < 0.001. Control: antibiotic only. CIT: citalopram.

Table 4. Diameter of inhibitory zones of selected antibiotics with increasing concentrations of venlafaxine (VF 75-1200 µg/mL) against *E. coli* ATCC 8739.

Antibiotic	Diameter of the inhibitory zone (mm); the mean ± SEM (n = 5)					
	Control	Antibiotic + VF 75 µg/mL	Antibiotic + VF 150 g/mL	Antibiotic + VF 300 µg/mL	Antibiotic + VF 600 µg/mL	Antibiotic + VF 1200 g/mL
Ciprofloxacin 5 µg	26.8 ± 6.26	28.2 ± 7.15	30.6 ± 6.50	32.2 ± 5.11	34.4 ± 5.54	36.2 ± 2.94
Levofloxacin 5 µg	25.4 ± 5.81	27 ± 5.83	28 ± 6.44	29.2 ± 4.38	32.2 ± 3.89	34.4 ± 2.88*
Norfloxacin 10 µg	22.6 ± 8.41	24 ± 8.63	25.6 ± 8.56	26.4 ± 7.02	29.6 ± 5.54	33 ± 3.00
Moxifloxacin 5 µg	29.4 ± 2.79	31.2 ± 2.77	31.8 ± 2.48	32.8 ± 2.58	34 ± 2.00*	35.8 ± 1.48**
Cefixime 5 µg	12.8 ± 11.51	16.8 ± 11.51	18.2 ± 2.55	19.6 ± 12.83	22.2 ± 8.87	21.80 ± 8.34
Cloxacillin 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	4.8 ± 1.024	7 ± 1.47	10 ± 2.47
Gentamycin 10 µg	25.8 ± 3.03	27.4 ± 3.84	29 ± 4.35	29.8 ± 4.60	30.6 ± 4.56	33.4 ± 4.66*

***:** Values significantly different as compared to antibiotic treatment group only, * p < 0.05, ** p < 0.01. Control: antibiotic only. CIT: citalopram.

better antibacterial as its has dual reuptake inhibitory mechanism (SNRI) in humans and could have inhibitory effect on efflux pumps. Being devoid of intrinsic antibacterial activity at concentrations range used (75-1200 µg/mL), it has significantly increased the activities of all antibiotics except cloxacillin in concentration dependent manner. *S. aureus* ATCC 6538 was resistant to cefixime alone but in the presence of VF its susceptibility was increased scoring an inhibitory zone of 12 mm (Table 2).

Resistance of *S. aureus* to various antibiotics develops due to the presence of NorA and other efflux pumps (32). Kaatz et al. reported that selective serotonin reuptake inhibitors has the ability to block this pump and reverse antibiotic resistance (33). The increase in susceptibility of *S. aureus* to the selected antibiotics may be due to physical interaction of CIT with NorA efflux pump and may be equally contributed to intrinsic antimicrobial activities of CIT. Also it was observed that there was attenuation of microbial growth in CIT added plates indicating its direct inhibitory effects on *S. aureus*. Our laboratory is still working to find out mechanism of CIT and VF induced bacterial death and their possible role as efflux pump inhibitors.

Effect of increasing concentrations of citalopram and venlafaxine on the susceptibility of *Escherichia coli*

Antibacterial activity of CIT and VF are summarized in Tables 3, 4. The combined antibacterial activity of CIT was more marked against Gram-positive bacteria (*S. aureus*) in comparison to Gram-negative (*E. coli and P. aeruginosa*). CIT at a concentration of 1250 µg/mL has significantly increased the antibacterial activity of selected drugs except cloxacillin and cefixime. *E. coli* initially resistant to cefixime become susceptible at 5000 µg/mL of CIT scoring an inhibitory zone of 10 mm. Though, this inhibitory effect was not statistically significant but might have an uncovered reversal mechanism involved there. The increase in antibacterial activity of VF was moderate against *E. coli*. Although diameter of inhibitory zones increased steadily along the concentration gradient but significant increase was observed only for levofloxacin, moxifloxacin and gentamicin at higher concentration. *E. coli* susceptibility to cloxacillin and cefixime was increased with the addition of VF to whom it was previously resistant. Among the two drugs, CIT was more effective in combination with antibiotics against *E. coli* as compared to VF. Researchers usually favor those compounds which are intrinsically inert but can synergize the antibacterial effect of other drugs. Thus, further studies on VF and derivation are necessary to uncover possible antibiotic-VF combinations and how to minimize neurological effects at effective concentrations.

Effect of increasing concentrations of citalopram and venlafaxine on the susceptibility of *Pseudomonas aeruginosa*

The vulnerability of *P. aeruginosa* to ciprofloxacin and norfloxacin was significantly increased with the addition of only 310 µg/mL of CIT, whereas levofloxacin and moxifloxacin exhibited synergistic interactions with CIT at 620 µg/mL (Tables 5 and 6). *P. aeruginosa* was resistant to cloxacillin initially, but with the addition of increasing concentrations of CIT its susceptibility was increased indicated by an increase in the diameter of inhibitory zone. Significant synergy between gentamicin and CIT was observed at 2500 µg/mL and resistance to cefixime was not affected at any concentration of CIT being used. VF at concentration of 600 µg/mL has significantly increased the inhibitory zones of ciprofloxacin, levofloxacin, norfloxacin and moxifloxacin against *P. aeruginosa*. Interestingly, its susceptibility to cloxacillin and cefixime has increased with the addition of VF. The exact mechanism of the antibacterial activity of these drugs is still not known, however it is hypothesized that as these compounds are pump inhibitors (SSRI & SNRI) in humans so they may inhibit the activity of bacterial efflux pumps. These compounds may also work by competing with the substrates thus reducing the function of these pumps. A more recent approach is that these inhibitor compounds have affinity for the substrates of MDR pumps. So, by forming an appropriate complex of antibiotic with these inhibitors can inhibit its efflux due to competitive inhibition or larger size.

CONCLUSIONS

The widespread multidrug resistance among pathogens necessitates the development of resistance modifying agents, which when combined with antimicrobial drugs will augment the activity of these agents and will prevent the emergence of acquired resistance. Drugs in combination (i.e., amoxicillin and clavulanic acid) will act synergistically to augment the effectiveness of drug therapy and give broad spectrum of activity. So it is required to interact antibiotics with non-antibiotic compounds to find out synergistic or potentiating characteristics of these drug combinations. These com-

Table 5. Diameter of inhibitory zones in mm of selected antibiotics with increasing concentrations of citalopram (310-5000 µg/mL) against *P. aeruginosa* ATCC 9027.

Antibiotic	Diameter of the inhibitory zone (mm); the mean ± SEM (n = 5)					
	Control	Antibiotic + CIT 310 µg/mL	Antibiotic + CIT 620 µg/mL	Antibiotic + CIT 1250 µg/mL	Antibiotic + CIT 2500 µg/mL	Antibiotic + CIT 5000 µg/mL
Ciprofloxacin 5 µg	25.2 ± 0.95	28 ± 1.41*	29 ± 1.41**	30.2 ± 0.95***	31.5 ± 1.00***	33.2 ± 1.50***
Levofloxacin 5 µg	24.5 ± 0.57	26.5 ± 1.00	28.5 ± 2.081**	30 ± 1.41***	31.2 ± 1.25***	34.5 ± 0.57***
Norfloxacin 10 µg	22.5 ± 1.00	25.7 ± 1.50*	27.5 ± 1.73***	28.5 ± 1.73***	30 ± 1.15***	31.7 ± 1.50***
Moxifloxacin 5 µg	25.7 ± 0.95	27.5 ± 1.29	29.5 ± 1.29**	30.5 ± 1.29***	30.5 ± 1.29***	32 ± 1.41***
Cefixime 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Cloxacillin 5 µg	0 ± 0.00	0 ± 0.00	0 ± 0.00	4.5 ± 1.00	7 ± 1.42	10.2 ± 1.50
Gentamycin 10 µg	24 ± 0.81	25.2 ± 0.95	27 ± 1.82	28 ± 1.82	28.7 ± 2.06*	30.2 ± 3.59**

*: Values significantly different as compared to antibiotic treatment group only, *p < 0.05, ** p < 0.01, *** p < 0.001. Control: antibiotic only. CIT: citalopram.

Table 6. Diameter of inhibitory zones of selected antibiotics with increasing concentrations of venlafaxine (VF 75-1200 µg/mL) against *P. aeruginosa* ATCC 9027.

Antibiotic	Diameter of the inhibitory zone (mm); the mean ± SEM (n = 5)					
	Control	Antibiotic + VF 75 µg/mL	Antibiotic + VF 150 g/mL	Antibiotic + VF 300 µg/mL	Antibiotic + VF 600 µg/mL	Antibiotic + VF 1200 g/mL
Ciprofloxacin 5 µg	27.25 ± 2.36	29 ± 2.58	30.2 ± 2.98	30.7 ± 2.87	33 ± 2.44*	35 ± 2.16**
Levofloxacin 5 µg	26.75 ±1.50	28.2 ± 0.95	28.7 ± 1.25	29.5 ± 1.91	31.5 ± 1.91**	33.7 ± 1.25***
Norfloxacin 10 µg	24 ±3.55	27 ± 4.24	27.5 ± 3.31	29 ± 3.82	31.7 ± 2.87*	33.5 ± 1.73**
Moxifloxacin 5 µg	27.25 ±0.50	30.2 ± 2.87	31.5 ± 3.31	32 ± 2.82	33.5 ± 2.38*	34.7 ± 2.50**
Cefixime 5 µg	0 ± 0.00	4.5 ± 1.00	7.2 ± 1.50	8.5 ± 0.50	10.7 ± 1.50	14 ± 1.50
Cloxacillin 5 µg	5 ± 0.70	6 ± 0.97	7.7 ± 0.67	9.2 ± 0.58	10.5 ± 0.79	11 ± 1.61
Gentamycin 10 µg	25.50 ± 3.31	29 ± 7.74	30.5 ± 8.18	32.2 ± 8.50	34.5 ± 7.93	37 ± 6.63

*: Values significantly different as compared to antibiotic treatment group only (Control), *p < 0.05, ** p < 0.01, *** p < 0.001. VF: venlafaxine.

binations will ultimately lead to broad spectrum drugs thus reducing cost and duration of antimicrobial drug therapy. The main reason behind this study was long term use of these antidepressant drugs and antimicrobial history of antidepressants and antipsychotics. From antibacterial studies of CIT and VF it is concluded that these possess antibacterial properties and both increased the activity of antibiotics in concentration dependent manner. VF being inactive intrinsically has potentiated antibacterial activities of antibiotics in combination while CIT has exhibited synergistic interactions with antibiotics. Resistance to some antibiotics is also reversed with the addition of these drugs. The daily recommended dose of CIT is 20-30 mg and can be increased up to 60 mg, whereas daily recommended dose of VF is 75-375 mg daily (34). As this is first screening of CIT and VF for possible antibacterial potentials, so high concentrations were also tested to find out useful range in relation to antimicrobial activity. However, from bioavailability point of view, any relevance between our *in vitro* results and *in vivo* performance is still not clear and may require further *in vivo* studies to investigate the mechanism of CIT and VF induced bacterial death. These antidepressants were very effective at higher concentration but achieving such concentrations clinically and neurological aspects will be a challenge. Further derivatization and use of bacteria containing molecularly characterized efflux pumps can provide more convincing results. Studies regarding structural information and possible efflux pumps inhibitory potential of these compounds in the presence and absence of known efflux pump inhibitors is in progress in our laboratory.

Acknowledgments

The authors sincerely thank Universal Pharmaceutical Labs and Usawa Pharmaceuticals, Pakistan, for providing citalopram and venlafaxine, respectively. We are also thankful to Cirin Pharmaceuticals, Pakistan, for providing ATCC strains of bacteria.

Competing interests

The authors declare that they have no competing interest.

REFERENCES

1. Walsh C.: Nature 406, 775 (2000).
2. Normark B.H., Normark S.: J. Intern. Med. 252, 91 (2002).
3. Marquez B.A.: Biochimie 87, 1137 (2005).
4. Martinez-Garriga B., Vinuesa T., Hernandez-Borrell J., Vivas M.: Int. J. Med. Microbiol. 297, 187 (2007).
5. Finlay J., Miller L., Poupard J.A.: J. Antimicrob. Chemother. 52, 18 (2003).
6. Renau T.E., Lemoine R.M.C.: Drugs Future 26, 1171 (2001).
7. Kaatz G.W.: Expert Opin. Emerg. Drugs 7, 223 (2002).
8. Kristiansen J.: APMIS. 30, 7 (1992).
9. Kristiansen J.E., Amaral L.: J. Antimicrob. Chemother. 40, 319 (1997).
10. Kruszewska H., Zaręba T., Tyski S.: Acta Pol. Pharm. Drug Res. 57, 117 (2000).
11. Munoz-Bellido J.L., Munoz-Criado S., Garca-Rodraguez J.A.: Int. J. Antimicrob. Agents 14, 177 (2000).
12. Coutaux A.F., Mooney J.J., Wirth D.F.: Antimicrob. Agents Chemother. 38, 1419 (1994).
13. Lass-Floral C., Dierich M.P., Fuchs D., Semenitz E., Jenewein I., Ledochowski M.: J. Antimicrob. Chemother. 48, 775 (2001).
14. Lass Floral C., Ledochowski M., Fuchs D., Speth C., Kacani L. et al.: FEMS Immunol. Med. Microbiol. 35, 11 (2003).
15. Kawase M., Motohashi N.: Current Drug Targets 4, 31 (2003).
16. Michalak K., Wesolowska O., Motohashi N., Molnar J., Hendrich A.B.: Current Drug Targets 7, 1095 (2006).
17. Amaral L., Kristiansen J.E.: Int. J. Antimicrob. Agents 14, 173 (2000).
18. Munoz-Bellido J., Munoz-Criado S., Garcia-Rodriguez J.: Int. J. Antimicrob. Agents 14, 177 (2000).
19. Kaatz G.W., Moudgal V.V., Seo S.M., Hansen J.B., Kristiansen J.E.: Int. J. Antimicrob. Agents 22, 254 (2003).
20. Wei P., Kaatz G.W., Kerns R.J.: Bioorg. Med. Chem. Lett. 14, 3093-3097 (2004).
21. Hendricks O., Butterworth T.S., Kristiansen J.E.: Int. J. Antimicrob. Agents 22, 262 (2003).
22. Katragadda S., Budda B., Anand B.S., Mitra A.K.: Expert Opin. Drug Deliv. 2, 683 (2005).
23. Barrow G.I., Feltham R.K.A.: in Cowan and Steel's Manual of the Identification of Medical Bacteria. pp. 50-59, 159-164, Cambridge University Press, Cambridge 1993.
24. Cederlund H., Mardh P.A.: J. Antimicrob. Chemother. 32, 355 (1993).
25. Cheesbrough M.: Medical Laboratory Manual for Tropical Countries. 2nd edn., Butterworth-Heinemann, Cambridge UK 1987.

26. Roberts W.K., Selitrennikoff C.P.: J. Gen. Microbiol. 134, 169 (1988).
27. Allen K.L., Molan P.C., Reid G.M.: J. Pharm. Pharmacol. 43, 817 (1991).
28. Cruickshank R., Duguid J.P., Marmion B.P., Swain R.H.A.: in Medical Microbiology, pp. 201-208, Churchill Livingstone, London 1989.
29. Standards, NCCLS.: Methods for dilution in antimicrobial susceptibility tests. Approved standard M2-A5. Villanova, PA 1993.
30. NCCLS.: Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A8. Wayne, PA 2003.
31. CLSI.: Performance Standards for Antimicrobial Susceptibility Testing. Eighteenth Informational Supplement, M100-S18; Wayne, PA, 2008.
32. Poole K.: Antimicrob. Agents Chemother. 44, 2233 (2000).
33. Kaatz G.W., Moudgal V.V., Seo S.M., Hansen J.B., Kristiansen J.E.: Int. J. Antimicrob. Agents 22, 254 (2003).
34. Martin J., BNF 57 March 2009 – British National Formulary., BMJ Group & RPS Press, London 2009.

Received: 25. 09. 2014