CHAPTER IV

RESULTS AND DISCUSSION

Laboratory Evaluations of Norfloxacin Against Bacterial Enteropathogens

1. Results of Antimicrobial Susceptibility Tests

Using the disc agar diffusion method mentioned before, 320 strains of bacterial enteropathogens, shown in table 2, were tested with norfloxacin 10 µg/disc, chloramphenicol 30 µg/disc, co-trimoxazole (SXT, TMP/SMT) 1.25 µg/23.75 µg per disc, ampicillin 10 µg/disc and tetracycline 30 µg/disc. The overall results are shown in table 3. The percentage of sensitive strains of these pathogens to the five antimicrobials tested are shown in table 4.

All tested strains showed to be susceptible to norfloxacin. The test included 5 strains of isolates (Salmonella group B 1, Shigella boydii 1, Shigella sonnei 1, and Shigella flexneri 2 strains) which were resistant to all other 4 antimicrobial agents tested. This was compared with the efficacy of four widely used antibiotics in gastrointestinal infections: i.e. chloramphenicol, co-trimoxazole, ampicillin and tetracycline.

The majority of the tested strains of Aeromonas, Plesiomonas, Salmonella group E, Shigella and Vibrio, except for Vibrio cholerae, were resistant to ampicillin. The susceptibility of Aeromonas caviae to chloramphenicol, co-trimoxazole, tetracycline and Aeromonas hydrophila to tetracycline were only 9 in 12 (75.0 %), 8 in 11 (72.7 %), 9 in 12 (75.0 %) and 19 in 25 (76.0 %) respectively. The susceptibility of Plesiomonas to tetracycline was only 62 in 92 (67.4 %). Most of Salmonella were resistant to tetracycline. The susceptibility of Salmonella group E to chloramphenicol was only 9 in 12 (75.0 %). The majority of Shigella were resistant to all the other four antimicrobial drugs tested. Shigella sonnei and Shigella flexneri were more susceptible to co-trimoxazole than Shigella boydii and Shigella dysentriae. The susceptibility of Shigella sonnei and Shigella flexineri to co-trimoxazole were 3 in 4 (75.0 %) and 16 in 18 (88.9 %) respectively. From the 320 strains of tested bacterial enteropathogens, the over all frequency of resistance were 5.4, 14.1, 27.3 and 69.1 % for co-trimoxazole, chloramphenicol, tetracycline and ampicillin respectively.

The results of the susceptibility of the antimicrobial agents tested are in line with the results of a study of Lolekha, S. and Patanacharoen, S. (35).

Table 2. The Bacterial Enteropathogens Used for the Antimicrobial Susceptability Test

PATHOGEN	NUMBER OF STRAIN(S)	PERCENT
Vibrionaceae		
Aeromonas	45	14.1
A. caviae	12	3.8
A. hydrophila	25	7.8
A. sobria	8	2.5
Plesiomonas	92	28.8
P. shigelloides	92	28.8
Vibrio	104	32.5
V. alginolyticus	10	3.1
V. cholerae	5	1.6
V. fluvialis	2	0.6
V. furnissii	4	1.3
V. parahaemolyticus	72	22.5
V. cholerae non-01	11	3.4
Enterobacteriaceae		
Salmonella	43	13.4
Salmonella group A	2	0.6
Salmonella group B	17	5.3
Salmonella group C	10	3.1
Salmonella group D	1	0.3
Salmonella group E	12	3.8
Salmonella group G	1	0.3
Shigella	31	9.7
Shigella boydii	7	2.2
Shigella dysenteriae	2	0.6
Shigella flexneri	18	5.6
Shigella sonnei	4	1.3
Edwardsiella	5	1.6
Edwardsiella tarda	5	1.6
TOTAL	320	100.0

Table 3. Results of the Antimicrobial Susceptability Test

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Aeromonas										
A. caviae										
Total Susceptible	12	100.0	9	75.0	8	72.7	0	0.0	9	75.0
Total Intermediate	0	0.0	1	8.3	0	0.0	0	0.0	1	8.3
Total Resistance	0	0.0	2	16.7	3	27.3	12	100.0	2	16.7
Total Tested	12		12		11		12		12	
A. hydrophila										
Total Susceptible	25	100.0	22	88.0	25	100.0	0	0.0	19	76.0
Total Intermediate	0	0.0	0	0.0	0	0.0	0	0.0	1	4.0
Total Resistance	0	0.0	3	12.0	0	0.0	25	100.0	5	20.0
Total Tested	25		25		25		25		25	
A. sobria										
Total Susceptible	8	100.0	8	100.0	8	100.0	2	25.0	7	87.5
Total Resistance	0	0.0	0	0.0	0	0.0	6	75.0	1	12.5
Total Tested	8		8		8		8		8	
Aeromonas tested										
Total Susceptible	45	100.0	39	86.7	41	93.2	2	4.4	35	77.8
Total Intermediate	0	0.0	1	2.2	0	0.0	0	0.0	2	4.4
Total Resistance	0	0.0	5	11.1	3	6.8	43	95.6	8	17.8
Total Tested	45		45		44		45		45	

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Plesiomonas										
P. shigelloides										
Total Susceptible	90	100.0	87	94.6	88	97.8	35	38.0	62	67.4
Total Intermediate	0	0.0	0	0.0	1	1.1	26	28.3	0	0.0
Total Resistance	0	0.0	5	5.4	1	1.1	31	33.7	30	32.6
Total Tested	90		92		90		92		92	

Table 3. (cont.)

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Vibrio										
V. alginolyticus										
Total Susceptible	10	100.0	10	100.0	10	100.0	2	20.0	10	100.0
Total Resistance	0	0.0	0	0.0	0	0.0	8	80.0	0	0.0
Total Tested	10		10		10		10		10	
V. cholerae										
Total Susceptible	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Total Tested	5		5		5		5		5	
V. fluvialis										
Total Susceptible	2	100.0	2	100.0	2	100.0	0	0.0	2	100.0
Total Resistance	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0
Total Tested	2		2		2		2		2	
V. furnissii										
Total Susceptible	4	100.0	4	100.0	4	100.0	1	25.0	4	100.0
Total Resistance	0	0.0	0	0.0	0	0.0	3	75.0	0	0.0
Total Tested	4		4		4		4		4	
V. parahaemolyticus										
Total Susceptible	72	100.0	70	100.0	72	100.0	2	2.8	71	100.0
Total Intermediate	0	0.0	0	0.0	0	0.0	3	4.2	0	0.0
Total Resistance	0	0.0	0	0.0	0	0.0	67	93.1	0	0.0
Total Tested	72		70		72		72		71	
V. chorerae non-01										
Total Susceptible	11	100.0	9	81.8	9	81.8	5	45.5	11	100.0
Total Intermediate	0	0.0	2	18.2	0	0.0	2	18.2	0	0.0
Total Resistance	0	0.0	0	0.0	2	18.2	4	36.4	0	0.0
Total Tested	11		11		11		11		11	
Vibrio tested										
Total Susceptible	104	100.0	100	98.0	102	98.1	15	14.4	103	100.0
Total Intermediate	0	0.0	2	2.0	0	0.0	5	4.8	0	0.0
Total Resistance	0	0.0	0	0.0	2	1.9	84	80.8	0	0.0
Total Tested	104		102		104		104		103	

Table 3. (cont.)

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Salmonella										
Salmonella group A										
Total Susceptible	2	100.0	2	100.0	2	100.0	2	100.0	1	50.0
Total Resistance	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0
Total Tested	2		2		2		2		2	
Salmonella group B										
Total Susceptible	17	100.0	16	94.1	15	88.2	16	94.1	10	58.8
Total Resistance	0	0.0	1	5.9	2	11.8	1	5.9	7	41.2
Total Tested	17		17		17		17		17	
Salmonella group C										
Total Susceptible	10	100.0	9	90.0	10	100.0	9	90.0	4	40.0
Total Intermediate	0	0.0	0	0.0	0	0.0	1	10.0	6	60.0
Total Resistance	0	0.0	1	10.0	0	0.0	0	0.0	0	0.0
Total Tested	10		10		10		10		10	
Salmonella group D										
Total Susceptible	1	100.0	1	100.0	1	100.0	1	100.0	0	0.0
Total Intermediate	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0
Total Tested	1		1		1		1		1	
Salmonella group E										
Total Susceptible	12	100.0	9	75.0	10	90.9	7	58.3	7	58.3
Total Resistance	0	0.0	3	25.0	1	9.1	5	41.7	5	41.7
Total Tested	12		12		11		12		12	
Salmonella group G										
Total Susceptible	1	100.0	1	100.0	1	100.0	1	100.0	1	100.0
Total Tested	1		1		1		1		1	
Salmonella tested										
Total Susceptible	43	100.0	38	88.4	39	92.9	36	83.7	23	53.5
Total Intermediate	0	0.0	0	0.0	0	0.0	1	2.3	7	16.3
Total Resistance	0	0.0	5	11.6	3	7.1	6	14.0	13	30.2
Total Tested	43		43		42		43		43	

Table 3. (cont.)

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Shigella										
Shigella boydii										
Total Susceptible	7	100.0	2	28.6	4	57.1	3	42.9	3	42.9
Total Resistance	0	0.0	5	71.4	3	42.9	4	57.1	4	57.1
Total Tested	7		7		7		7		7	
Shigella dysenteriae										
Total Susceptible	2	100.0	0	0.0	1	50.0	1	50.0	0	0.0
Total Resistance	0	0.0	2	100.0	1	50.0	1	50.0	2	100.0
Total Tested	2	100.0	2		2		2		2	
Shigella flexneri										
Total Susceptible	18	100.0	2	11.1	16	88.9	2	11.1	1	5.6
Total Resistance	0	0.0	16	88.9	2	11.1	16	88.9	17	94.4
Total Tested	18		18		18		18		18	
Shigella sonnei										
Total Susceptible	4	100.0	0	0.0	3	75.0	0	0.0	0	0.0
Total Resistance	0	0.0	4	100.0	1	25.0	4	100.0	4	100.0
Total Tested	4		4		4		4		4	
Shigella tested										
Total Susceptible	31	100.0	4	12.9	24	77.4	6	19.4	4	12.9
Total Resistance	0	0.0	27	87.1	7	22.6	25	80.6	27	87.1
Total Tested	31		31		31		31		31	

Table 3. (cont.)

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Edwardsiella										
Edwardsiella tarda										
Total Susceptible	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Total Tested	5		5		5		5		5	

PATHOGEN	NORFLOXACIN		CHLORAMPHENICOL		CO-TRIMOXAZOLE		AMPICILLIN		TETRACYCLINE	
	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT
Total Tested Pathogens										
Total Susceptible	318	100.0	273	85.8	299	94.6	99	30.9	232	72.7
Total Intermediate	0	0.0	3	0.9	1	0.3	57	17.8	9	2.8
Total Resistance	0	0.0	42	13.2	16	5.1	164	51.3	78	24.5
Total Tested	318		318		316		320		319	

Table 4. Summary of the Antibiotics Susceptability Test

Antibiotic	Susceptible / tested	Intermediate or resistant strains
Norfloxacin	318/318 (100 %)	None
Chloramphenicol	273/318 (85.9 %)	A. hydrophila (3), A. species (3), P. shigelloides (5), V. cholerae non-01 (2), Sal. group B (1), Sal. species (4), Sh. flexneri (16), Sh. species (11)
Co-trimoxazole	299/316 (94.6 %)	A. species (3), P.shigelloides (2), V.cholerae non-01 (2), Sal. group B (2), Sal. species (1), Sh. flexneri (2), Sh. species (5)
Ampicillin	99/320 (30.9 %)	A. hydrophila (25), A. species (18), P. shigelloides (57), V. parahaemolyticus (70), V. choleae non-01 (6), V. species (13), Sal. group B (1), Sal. species (6), Sh. flexneri (16), Sh. species (9)
Tetracycline	232/319 (72.7 %)	A. hydrophila (6), A. species (4), P. shigelloides (30), Sal. group B (7), Sal. species (13), Sh. flexneri (17), Sh. species (10)

2. Results of Determination of Minimal Inhibitory Concentration (MIC)

One hundred and seventy-six strains of bacterial enteropathogens, shown in table 5, were tested. The results of the test showed that all the tested strains were very susceptible to norfloxacin (table 6, 7). 27 in 28 (96.4 %) of Salmonella and 55 in 57 (96.5 %) of Vibrio tested were inhibited at MIC 0.125 and 0.25 mg/L respectively. All strains of Edwardsiella, Plesiomonas, Aeromonas, Shigella, Salmonella and Vibrio were inhibited at MIC 0.016, 0.031, 0.125, 0.125, 0.25 and 0.5 mg/L respectively.

The MIC_{50} and MIC_{90}

The activities of norfloxacin against bacterial enteropathogens are shown in table 8 and figure 2 as the relationship between the cumulative percentage of the inhibited pathogens and log MIC values. By interpolating the graph to meet the minimum inhibitory concentrations from 50 and 90 cumulative percentage inhibited, the MIC_{50} and MIC_{90} obtained are shown in table 8.

Table 5. Bacterial Enteropathogens Used for Determination of Minimal Inhibitory Concentration (MIC)

PATHOGEN	NUMBER OF STRAIN(S)	PERCENT
Vibrionaceae		
Aeromonas	26	14.8
A. caviae	7	4.0
A. hydrophila	15	8.5
A. sobria	4	2.3
Plesiomonas	45	25.6
P. shigelloides	45	25.6
Vibrio	57	32.4
V. alginolyticus	5	2.8
V. cholerae	3	1.7
V. furnissii	4	2.3
V. parahaemolyticus	39	22.2
V. cholerae non-01	6	3.4
Enterobacteriaceae		
Salmonella	28	15.9
Salmonella group A	1	0.6
Salmonella group B	10	5.7
Salmonella group C	8	4.5
Salmonella group E	8	4.5
Salmonella group G	1	0.6
Shigella	16	9.1
Shigella boydii	1	0.6
Shigella dysenteriae	2	1.1
Shigella flexneri	10	5.7
Shigella sonnei	3	1.7
Edwardsiella	4	2.3
Edwardsiella tarda	4	2.3
TOTAL	176	100.0

Table 6. The MICs of Norfloxacin against Bacterial Enteropathogens

MIC (ug/L)	0.016		0.031		0.062		0.125		0.25		0.5		
PATHOGEN	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	NUMBER OF STRAINS	PER-CENT	TOTAL
Aeromonas													
A. caviae	0	0.0	2	28.6	3	42.9	2	28.6	0	0.0	0	0.0	7
A. hydrophila	0	0.0	3	20.0	3	20.0	9	60.0	0	0.0	0	0.0	15
A. sobria	0	0.0	2	50.0	2	50.0	0	0.0	0	0.0	0	0.0	4
TOTAL Aeromonas	0	0.0	7	26.9	8	30.8	11	42.3	0	0.0	0	0.0	26
Plesiomonas													
P. shigelloides	0	0.0	45	100.0	0	0.0	0	0.0	0	0.0	0	0.0	45
Vibrio													
V. alginolyticus	0	0.0	0	0.0	0	0.0	3	60.0	2	40.0	0	0.0	5
V. cholerae	1	33.3	2	66.7	0	0.0	0	0.0	0	0.0	0	0.0	3
V. furnissii	0	0.0	0	0.0	0	0.0	2	50.0	1	25.0	1	25.0	4
V. parahaemolyticus	0	0.0	4	10.3	6	15.4	24	61.5	4	10.3	1	2.6	39
V. cholerae non-01	6	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6
TOTAL Vibrio	7	12.3	6	10.5	6	10.5	29	50.9	7	12.3	2	3.5	57
Salmonella													
Salmonella group A	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1
Salmonella group B	0	0.0	0	0.0	6	60.0	4	40.0	0	0.0	0	0.0	10
Salmonella group C	0	0.0	0	0.0	8	100.0	0	0.0	0	0.0	0	0.0	8
Salmonella group E	0	0.0	0	0.0	0	0.0	7	87.5	1	12.5	0	0.0	8
Salmonella group G	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	1
TOTAL Salmonella	0	0.0	1	3.6	15	53.6	11	39.3	1	3.6	0	0.0	28
Shigella													
Shigella boydii	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	1
Shigella dysenteriae	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0	2
Shigella flexneri	0	0.0	2	20.0	4	40.0	4	40.0	0	0.0	0	0.0	10
Shigella sonnei	0	0.0	3	100.0	0	0.0	0	0.0	0	0.0	0	0.0	3
TOTAL Shigella	0	0.0	5	31.3	4	25.0	7	43.8	0	0.0	0	0.0	16
Edwardsiella													
Edwardsiella tarda	4	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4

Table 7. The Summarized Data of the MICs of Norfloxacin Against Bacterial Enteropathogens Tested

PATHOGEN	NO. OF STRAINS	MODAL MIC (mg/L)	NO. OF MODE	% OF MODE	MIC RANGE (mg/L)
A. hydrophila	15	0.125	9	60.0	0.031-0.125
A. species	11	0.062	5	45.5	0.031-0.125
P. shigelloides	45	0.031	45	100.0	0.031-0.031
V. parahaemolyticus	39	0.125	24	61.5	0.031-0.5
V. cholera non-01	6	0.016	6	100.0	0.016-0.016
V. species	12	0.125	5	41.7	0.016-0.5
Salmonella group B	10	0.062	6	60.0	0.062-0.125
Salmonella species	18	0.062	9	50.0	0.031-0.25
Shigella flexneri	10	0.062, 0.125	4, 4	40.0, 40.0	0.031-0.125
Shigella species	6	0.031, 0.125	3, 3	50.0, 50.0	0.031-0.125
Edwardsiella tarda	4	0.016	4	100.0	0.016-0.016

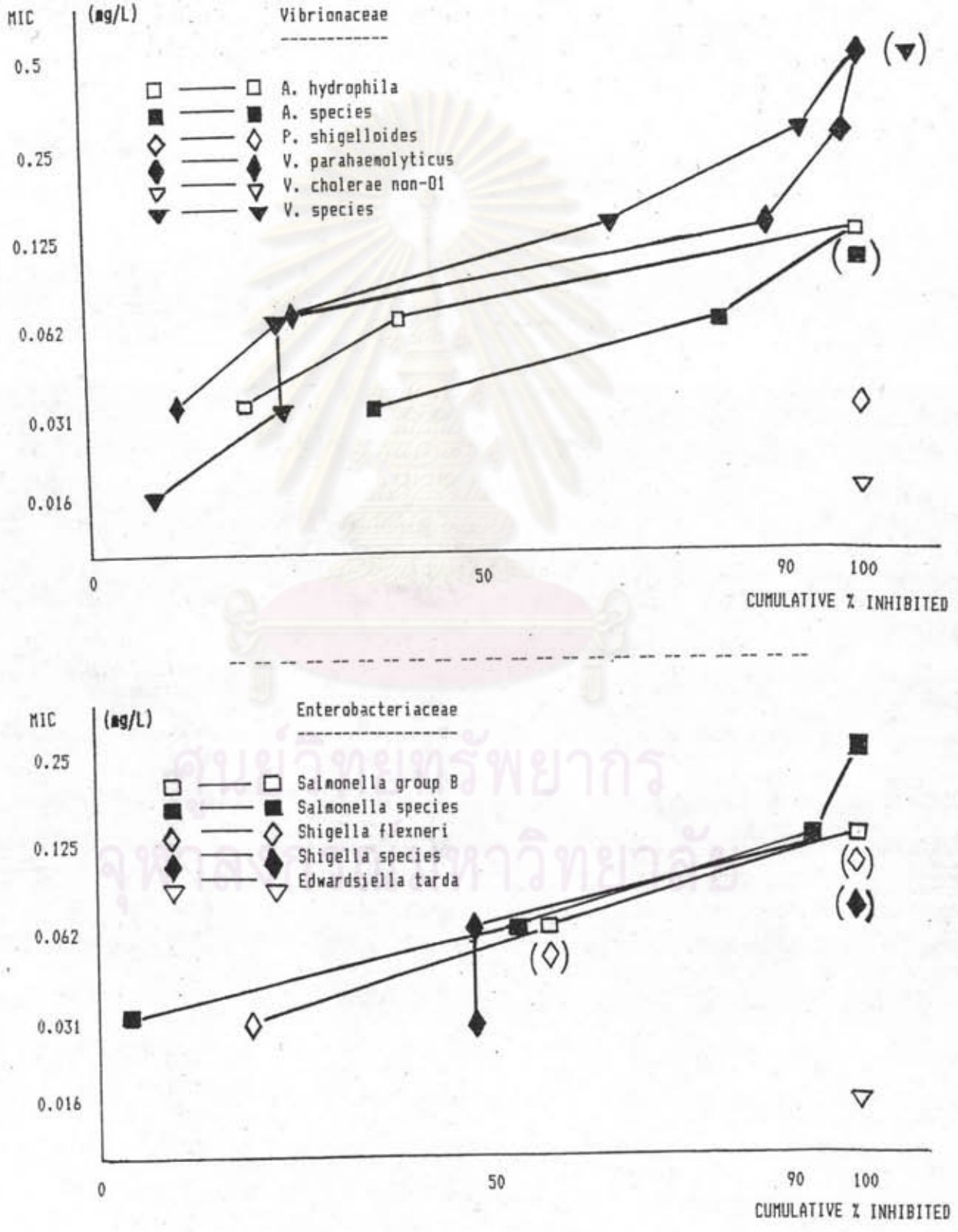
Table 8. The Activities of Norfloxacin (MIC_{50} and MIC_{90}) Against Bacterial Enteropathogens

PATHOGEN	MIC (mg/L)	NO. OF STRAINS (%)	CUMULATIVE % INHIBITED	MIC_{50} (mg/L)	MIC_{90} (mg/L)
A. hydrophila	0.031	3 (20.00)	20.00	0.069	0.11
	0.062	3 (20.00)	40.00		
	0.125	9 (60.00)	100.00		
A. species	0.031	4 (36.36)	36.36	0.039	0.085
	0.062	5 (45.45)	81.81		
	0.125	2 (18.19)	100.00		
P. shigelloides	0.031	45 (100)	100.00		
V. parahaemolyticus	0.031	4 (10.26)	10.26	0.082	0.145
	0.062	6 (15.38)	25.64		
	0.125	24 (61.54)	87.18		
	0.25	4 (10.26)	97.44		
	0.5	1 (2.56)	100.00		
V. cholerae non-01	0.016	6 (100)	100.00		
V. species	0.016	1 (8.33)	8.33	0.093	0.24
	0.031	2 (16.67)	25.00		
	0.062	0 (0.00)	25.00		
	0.125	5 (41.67)	66.67		
	0.25	3 (25.00)	91.67		
	0.5	1 (8.33)	100.00		

Table 8. (cont.)

PATHOGEN	MIC (mg/L)	NO. OF STRAINS (%)	CUMULATIVE % INHIBITED	MIC 50 (mg/L)	MIC 90 (mg/L)
Salmonella group B	0.062	6 (60.00)	60.00		0.105
	0.125	4 (40.00)	100.00		
Salmonella species	0.031	1 (5.55)	5.55	0.058	0.115
	0.062	9 (50.00)	55.55		
	0.125	7 (38.90)	94.45		
	0.25	1 (5.55)	100.00		
Shigella flexneri	0.031	2 (20.00)	20.00	0.052	0.105
	0.062	4 (40.00)	60.00		
	0.125	4 (40.00)	100.00		
Shigella species	0.031	3 (50.00)	50.00	0.031	0.11
	0.062	0 (0.00)	50.00		
	0.125	3 (50.00)	100.00		
Edwardsiella tarda	0.016	4 (100)	100.00		

Figure 2. The Activities of Norfloxacin Against Bacterial Enteropathogens



The minimum inhibitory concentrations (MICs) of norfloxacin against the bacterial enteropathogens tested were very low. The MIC_{50} and MIC_{90} of Vibrionaceae tested were 0.039-0.093 and 0.085-0.24 mg/L for Aeromonas and Vibrio respectively. All Plesiomonas tested were inhibited at 0.031 mg/L. The MIC_{50} and MIC_{90} of Enterobacteriaceae tested were 0.031-0.058 and 0.015-0.115 mg/L for Salmonella and Shigella respectively. These results are in line with the results of the other studies (see page 9).

Bacteria could be considered susceptable when MICs are $\leq$ 4 mg/L and resistant when MICs are $\geq$ 15 mg/L (87). Our in vitro data suggested that oral norfloxacin might be adequate in treating the infections of bacterial enteropathogens since norfloxacin reaches high level in stool that surpass the MIC for most enteropathogens, with concentration of 600 mg/L of stool after a single oral dose of 400 mg (34).

Other reports suggested that the activity of norfloxacin was not affected by the type of medium, pH, or inoculum size (24,36,39,57). The MICs for norfloxacin obtained by broth dilution were slightly higher than those obtained by agar dilution (36). The minimal bactericidal concentrations (MBCs) of norfloxacin were only slightly higher than the MICs, even at high inocula indicated that the development of resistance against this drug might be less common (36,39,57).

These in vitro results supported the idea of this research which tried to discover the more active compound against the bacterial enteropathogens.

Clinical Evaluations of Norfloxacin in Acute Bacterial Diarrhoea

1. Patient Descriptions

Patient descriptions are shown in table 9. A total of 260 patients were recruited into the study from February 1986 to July 1987. The gastrointestinal infection could not be bacteriologically verified in 81 out of 260 patients (31.2 %). This is in line with practical clinical experience (35). These patients were excluded from efficacy evaluation together with 25 patients who had another protocol violation.

A total of 154 patiets were considered to be eligible for efficacy and safety evaluations. 49, 50 and 55 out of 154 patients were treated with norfloxacin and placebo (NFX group), co-trimoxazole and placebo (SXT group) and placebo (placebo group) respectively. There were some differences in the sex distribution among these 3 groups, but were not considered to affect the severity of disease or the outcome of treatments. Age and weight distribution among these 3 groups were comparable with the average age 37, 35 and 34 years and average weight of 51.8, 47.0 and 47.7 kg in NFX group, SXT group and placebo group respectively. The comparable amount of the patients

in these 3 groups had taken drugs to alleviate their diarrhoeal symptoms before they attended to the hospital, the fact that might interfere with the baseline data.

Table 9. Patient Descriptions (Number of Patients)

Total recruited	260
Negative initial culture	81
Other antibiotic drug(s) taken (or suspected)	21
Another protocol violation	4
Total excluded	106
Valid for efficacy and safety evaluations.	154

	NFX group	SXT group	placebo group
Valid for evaluations	49	50	55
- Sex distribution (male / female)	24 / 25	33 / 17	27 / 28
- Age distribution			
16-30 years	20	22	24
31-50 years	19	20	26
51-65 years	10	8	5
- Weight distribution			
30-50 kg	18	20	26
51-65 kg	25	24	24
>65 kg	4	0	1
unknown	2	6	4
- Medication before attending this study	44 (89.8%)	40 (80.0%)	46 (83.6%)

2. Causative Organisms

Bacterial enteropathogens found prior to treatment initiation and the distribution of these pathogens are shown in table 10 and table 11 respectively.

Among the 154 patients, 235 strains of bacterial enteropathogens were isolated initially. Vibrio parahemolyticus and Plesiomonas shigelloides were the two most prevalent pathogens found, with frequencies of 28.5 and 24.7 % respectively. Other important species were Escherichia coli (8.9 %), Aeromonas hydrophila (8.1 %), Shigella species (7.7 %) and Salmonella species (7.2 %). A single strain was found in 60.4 % of these patients, whereas 29.9, 7.1 and 2.6 % of these patients had two, three and four or more strains isolated initially respectively.

Table 10. Bacterial Enteropathogens Found Prior to Treatment Initiation. (Analysable Patients with Several Strains Appear Several Times)

PATHOGEN	NFX GROUP		SXT GROUP		PLACEBO GROUP		TOTAL	
	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
Aeromonas	6	7.7	5	7.1	17	19.5	28	11.9
A. caviae	1	1.3	0	0.0	4	4.6	5	2.1
A. hydrophila	4	5.1	5	7.1	10	11.5	19	8.1
A. sobria	1	1.3	0	0.0	3	3.4	4	1.7
Plesiomonas	22	28.2	17	24.3	19	21.8	58	24.7
P. shigelloides	22	28.2	17	24.3	19	21.8	58	24.7
Vibrio	29	37.2	29	41.4	28	32.2	86	36.6
V. alginolyticus	3	3.8	2	2.9	2	2.3	7	3.0
V. cholerae	0	0.0	3	4.3	1	1.1	4	1.7
V. fluvialis	0	0.0	1	1.4	0	0.0	1	0.4
V. furnissii	1	1.3	0	0.0	1	1.1	2	0.9
V. parahaemolyticus	23	29.5	22	31.4	22	25.3	67	28.5
V. cholerae non-01	2	2.6	1	1.4	2	2.3	5	2.1
Salmonella	6	7.7	5	7.1	6	6.9	17	7.2
Salmonella group A	0	0.0	0	0.0	1	1.1	1	0.4
Salmonella group B	2	2.6	3	4.3	2	2.3	7	3.0
Salmonella group C	1	1.3	1	1.4	2	2.3	4	1.7
Salmonella group D	1	1.3	0	0.0	0	0.0	1	0.4
Salmonella group E	2	2.6	0	0.0	1	1.1	3	1.3
Salmonella group G	0	0.0	1	1.4	0	0.0	1	0.4
Shigella	5	6.4	3	4.3	10	11.5	18	7.7
Shigella boydii	1	1.3	0	0.0	0	0.0	1	0.4
Shigella dysenteriae	0	0.0	0	0.0	1	1.1	1	0.4
Shigella flexneri	4	5.1	3	4.3	6	6.9	13	5.5
Shigella sonnei	0	0.0	0	0.0	3	3.4	3	1.3
Edwardsiella	1	1.3	0	0.0	2	2.3	3	1.3
Edwarssiella tarda	1	1.3	0	0.0	2	2.3	3	1.3
Escherichia	8	10.3	9	12.9	4	4.6	21	8.9
Escherichia coli								
- Enteroinvasive	3	3.8	1	1.4	2	2.3	6	2.6
- Enterotoxigenic								
- Heat labile toxin	2	2.6	3	4.3	0	0.0	5	2.1
- Heat stable toxin	3	3.8	5	7.1	2	2.3	10	4.3
Campylobacter	1	1.3	2	2.9	1	1.1	4	1.7
Campylobacter jejuni	1	1.3	2	2.9	1	1.1	4	1.7
TOTAL	78	33.2	70	29.8	87	37.0	235	100

Table 11. Distribution of the Number of Strains Found Prior to Treatment Initiation

PRIOR TO TREATMENT (DAY 0)	DRUG							
	NFX GROUP		SXT GROUP		PLACEBO GROUP		TOTAL	
NUMBER OF STRAIN(S)	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
1	26	53.0	35	70.0	32	58.2	93	60.4
2	18	36.8	11	22.0	17	30.9	46	29.9
3	4	8.2	3	6.0	4	7.3	11	7.1
4	1	2.0	1	2.0	1	1.8	3	1.9
5	0	0	0	0.0	1	1.8	1	0.7
TOTAL	49	31.8	50	32.5	55	35.7	154	100

3. Bacteriological Response

Bacteriological Results

The bacteriological results day by day of patients' stool cultures are shown in table 12. The frequency of the patients with free bacterial enteropathogen in the stool samples in NFX group was higher than both SXT group and placebo group, whereas the frequency in SXT group was higher than placebo group in all three days of treatment. One patient in NFX group had a relapse of pathogen on day 2, whereas higher frequencies of relapse and reinfection were registered in both SXT group and placebo group during three days of treatment.

Table 12. Bacteriological Results

DAY 1	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
BACTERIOLOGICAL RESULT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
ELIMINATION (ELIM)	44	89.7	20	40.0	19	34.5	83	53.8
PERSISTENCE (PERS)	5	10.2	22	44.0	23	41.8	50	32.4
REINFECTION (REIN)	0	0	4	8.0	5	9.1	9	5.8
PERS-REIN	0	0	4	8.0	8	14.5	12	7.8
TOTAL	49	31.8	50	32.5	55	35.7	154	100

DAY 2	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
BACTERIOLOGICAL RESULT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
ELIMINATION (ELIM)	48	98.0	41	82.0	28	50.9	117	75.9
PERSISTENCE (PERS)	0	0.0	6	12.0	14	25.4	20	12.9
RELAPSE (RELAPSE)	1	2.0	1	2.0	1	2.1	3	1.9
REINFECTION (REIN)	0	0.0	1	2.0	8	14.5	9	5.8
PERS-RELAPSE	0	0.0	0	0.0	1	1.8	1	0.6
PERS-REIN	0	0.0	1	2.0	1	1.8	2	1.3
RELAPSE-REIN	0	0.0	0	0.0	2	3.6	2	1.3
TOTAL	49	31.8	50	32.5	55	35.7	154	100

Table 12. (cont.)

DAY 3	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
BACTERIOLOGICAL RESULT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
ELIMINATION (ELIM)	46	100	40	83.3	27	49.1	113	75.8
PERSISTENCE (PERS)	0	0	2	4.2	11	20.0	13	8.7
RELAPSE (RELAPSE)	0	0	2	4.2	10	18.2	12	8.1
REINFECTION (REIN)	0	0	3	6.3	5	9.1	8	5.4
PERS-RELAPSE	0	0	0	0.0	1	1.8	1	0.7
PERS-REIN	0	0	0	0.0	1	1.8	1	0.7
RELAPSE-REIN	0	0	1	2.1	0	0.0	1	0.7
TOTAL	46	30.9	48	32.2	55	36.9	149	100

NOTE: MISSING DATA: NFX GROUP = 3 IN 49 (6.1 %), SXT GROUP = 2 IN 50 (4.0 %)

Time to Elimination (Bacteriological Survival Analysis)

Day(s) to end of infection are shown in table 13. 41, 45 and 46 out of 46 patients in NFX group (89.1, 97.8 and 100 % respectively) were bacteriologically cured after 1, 2 and 3 day(s) of treatment respectively, whereas 17, 34 and 40 out of 48 patients in SXT group (35.4, 70.8 and 83.3 % respectively) and 8, 18 and 27 of 55 patients in placebo group (14.5, 32.7 and 49.1 % respectively) were bacteriologically cured after 1, 2 and 3 day(s) of treatment respectively.

Table 13. Time to Elimination (Bacteriological Survival Analysis)

DAY(S) TO END OF INFECTION	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
DAY 1	41	89.1	17	35.4	8	14.5	66	44.3
DAY 2	4	8.7	17	35.4	10	18.2	31	20.8
DAY 3	1	2.2	6	12.5	9	16.4	16	10.7
NO ELIMINATION (WITHIN 6 DOSES)	0	0.0	8	16.7	28	50.9	36	24.2
TOTAL	46	30.9	48	32.2	55	36.9	149	100

Comparing the efficacy to eliminate the enteropathogens statistically, NFX group was more effective than both SXT group and placebo group in all three treatment days. The difference between the efficacy of NFX group and SXT group are shown by the p values of < 0.001, < 0.01 and < 0.1 on day 1, 2 and 3 respectively, whereas the difference between the efficacy of NFX group and placebo group are shown by the p values of < 0.001 on all three treatment days. Comparing SXT group to placebo group, SXT group was more effective than placebo group as shown by the p values of < 0.05, < 0.001 and < 0.001 on day 1, 2 and 3 respectively.

Bacteriological Cure Rate on Follow up Day

The bacteriological evaluations on the follow up day are shown in table 14. A total of 112 from 154 patients (72.7 %) returned to the hospital for a latter follow up on day 10-15. The frequencies of elimination, persistence, relapse and reinfection were not statistically different in all three treatment groups ($p > 0.05$). The rate of reinfections without symptoms were high in all three treatment groups (see page 69).

Table 14. Bacteriological Effect on Follow up Day

FOLLOW-UP DAY	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
BACTERIOLOGICAL RESULT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
ELIMINATION (ELIM)	29	82.9	25	65.8	31	79.4	85	75.9
PERSISTENCE (PERS)	0	0	1	2.6	1	2.6	2	1.8
RELAPSE (RELAPSE)	0	0	1	2.6	1	2.6	2	1.8
REINFECTION (REIN)	6	17.1	10	26.3	5	12.8	21	18.7
RELAPSE-REIN	0	0	1	2.6	1	2.6	2	1.8
TOTAL	35	31.3	38	33.9	39	34.8	112	100

NOTE: MISSING FOLLOW-UP DATA: NFX GROUP = 14 IN 49 (28.6 %), SXT GROUP = 12 IN 50 (24.0 %),

PLACEBO GROUP = 16 IN 55 (29.1 %)

TOTAL MISSING FOLLOW-UP DATA = 42 (27.3 %)

4. Frequencies and Characteristics of Stools

Frequencies and Characteristics of Stools

Frequencies and characteristics of the patients' stools day by day are shown in table 15. The proportions of the patients with watery stools at inclusion were similar in all three treatment groups, 149 out of 154 (96.8 %). During the first 24 hours of treatment only little changes were observed. However, a substantial decrease of the patients with watery stools took place during the second day of treatment in all three treatment groups. On the third day only 2 out of 49 (4.1 %), 4 out of 50 (8.0 %) and 6 out of 55 (10.9 %) patients in NFX group, SXT group and placebo group respectively still had abnormal stool (see page 33).

Table 15. Frequencies and Characteristics of Stools

DAY 0	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
STOOL CONSISTENCY (STOOL FREQUENCY)	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
WATERY (4 - 6)	18	36.7	20	40.0	25	45.4	63	40.9
WATERY (7 - 9)	9	18.4	7	14.0	10	18.1	26	16.9
WATERY (⩾ 10)	21	42.9	22	44.0	17	30.9	60	39.0
LOOSE (4 - 6)	0	0.0	1	2.0	1	1.81	2	1.3
LOOSE (7 - 9)	1	2.0	0	0.0	1	1.81	2	1.3
LOOSE (⩾ 10)	0	0.0	0	0.0	1	1.81	1	0.6
TOTAL	49	31.8	50	32.5	55	35.7	154	100

Table 15. (cont.)

DAY 1	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
STOOL CONSISTENCY (STOOL FREQUENCY)	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
WATERY (1 - 3)	9	18.4	13	26.0	11	20.0	33	21.4
WATERY (4 - 6)	11	22.4	14	28.0	21	38.2	46	29.9
WATERY (7 - 9)	10	20.4	8	16.0	7	12.7	25	16.2
WATERY (⟩ 10)	7	14.3	10	20.0	10	18.2	27	17.5
LOOSE (1 - 3)	5	10.2	2	4.0	2	3.6	9	5.8
LOOSE (4 - 6)	6	12.2	3	6.0	2	3.6	11	7.1
LOOSE (7 - 9)	1	2.0	0	0.0	0	0.0	1	0.6
LOOSE (⟩ 10	0	0.0	0	0.0	1	1.8	1	0.6
NONE	0	0.0	0	0.0	1	1.8	1	0.6
TOTAL	49	31.8	50	32.5	55	35.7	154	100

Table 15. (cont.)

DAY 2	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
STOOL CONSISTENCY (STOOL FREQUENCY)	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
WATERY (1 - 3)	4	8.2	0	0.0	5	9.1	9	5.8
WATERY (4 - 6)	3	6.1	2	4.0	1	1.8	6	3.9
WATERY (7 - 9)	0	0.0	2	4.0	0	0.0	2	1.3
WATERY (⩾ 10)	3	6.1	2	4.0	3	5.5	8	5.2
LOOSE (1 - 3)	23	46.9	19	38.0	23	41.8	65	42.2
LOOSE (4 - 6)	8	16.3	3	6.0	4	7.3	15	9.7
LOOSE (7 - 9)	0	0.0	1	2.0	1	1.8	2	1.3
LOOSE (⩾ 10)	0	0.0	0	0.0	1	1.8	1	0.6
WELL-FORMED (1 - 3)	7	14.3	12	24.0	13	23.6	32	20.8
WELL-FORMED (4 - 6)	0	0.0	2	4.0	0	0.0	2	1.3
NONE	1	2.0	7	14.0	4	7.3	12	7.8
TOTAL	49	31.8	50	32.5	55	35.7	154	100

Table 15. (cont.)

DAY 3	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
STOOL CONSISTENCY (STOOL FREQUENCY)	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
WATERY (1 - 3)	0	0.0	1	2.0	0	0.0	1	0.6
WATERY (4 - 6)	0	0.0	0	0.0	1	1.8	1	0.6
WATERY (7 - 9)	1	2.0	0	0.0	0	0.0	1	0.6
WATERY (⟩, 10)	0	0.0	1	2.0	3	5.5	4	2.6
LOOSE (1 - 3)	13	26.5	10	20.0	8	14.5	31	20.1
LOOSE (4 - 6)	1	2.0	1	2.0	0	0.0	2	1.3
LOOSE (7 - 9)	0	0.0	0	0.0	1	1.8	1	0.6
WELL-FORMED (1 - 3)	31	63.3	31	62.0	37	67.3	99	64.3
WELL-FORMED (4 - 6)	0	0.0	1	2.0	1	1.8	2	1.3
NONE	3	6.1	5	10.0	4	7.3	12	7.8
TOTAL	49	31.8	50	32.5	55	35.7	154	100

Time to Recover (Clinical Survival Analysis)

Day(s) to recover are shown in table 16. 3, 30 and 47 out of 49 patients in NFX group (6.1, 61.2 and 95.9 % respectively) had normal stools after 1, 2 and 3 day(s) of treatment respectively, whereas 2, 37 and 46 out of 50 patients in SXT group (4.0, 74.0 and 92.0 % respectively) and 2, 39 and 49 out of 55 patients in placebo group (3.6, 70.9 and 89.1 % respectively) had normal stools after 1, 2 and 3 day(s) of treatment respectively.

Table 16. Day(s) to Recover (Clinical Survival Analysis)

DAY(S) TO RECOVER	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
DAY 1	3	6.1	2	4.0	2	3.6	7	4.5
DAY 2	27	55.1	35	70.0	37	67.3	99	64.3
DAY 3	17	34.7	9	18.0	10	18.2	36	23.4
NOT RECOVER (WITHIN 6 DOSE)	2	4.1	4	8.0	6	10.9	12	7.8
TOTAL	49	31.8	50	32.5	55	35.7	154	100

Comparing the efficacy to improve frequency and consistency of the pateints' stools, the efficacy of these three groups were not statistically different in all three treatment days ($p > 0.1$). After three days of treatment,

the frequencies of the patients who still had diarrhoea in these three groups were not statistically different ($p > 0.1$).

Cure Rate on Follow up Day (Day 10-15)

The frequency and consistency of the patients' stools on the follow up day (table 17) showed that the stools of all the patients in all three treatment groups were normal.

Table 17. Frequencies and Characteristics of Stools on Follow up Day

FOLLOW-UP DAY	DRUG						TOTAL	
	NFX GROUP		SXT GROUP		PLACEBO GROUP			
STOOL CONSISTENCY (STOOL FREQUENCY)	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT	NUMBER OF PATIENTS	PER-CENT
WATERY	0	0.0	0	0.0	0	0.0	0	0.0
LOOSE (1-3)	2	9.1	3	13.0	1	4.0	6	8.6
WELL-FORMED	20	90.9	20	87.0	24	96.0	64	91.4
TOTAL	22	31.4	23	32.9	25	35.7	70	100

NOTE: MISSING FOLLOW-UP DATA: NFX GROUP = 27 IN 49 (55.1 %), SXT GROUP = 27 IN 50 (54.0 %)

PLACEBO GROUP = 30 IN 55 (54.5 %)

TOTAL MISSING FOLLOW-UP DATA = 84 IN 154 (54.5 %)

5. Fever Response

6 out of 49 (12.2 %), 8 out of 50 (16.0 %) and 6 out of 55 (10.1 %) patients in NFX group, SXT group and placebo group respectively had used antipyretic drugs, the fact that might interfere with the baseline data. However, 3 out of 6 (50.0 %), 2 out of 8 (25.0 %) and 3 out of 6 (50.0 %) patients who had used antipyretic drugs in each group were febrile (body temperature $\geq$ 38 ^{o}C) on admission day. (table 18).

The results of fever response are shown in table 18. 26 out of 49 (53.0 %), 22 out of 50 (44.0 %) and 26 out of 55 (47.3 %) patients in NFX group, SXT group and placebo group were febrile at the time of admission and on day 1. 25 out of 26 (96.2 %), 22 out of 22 (100 %) and 24 out of 26 (92.3 %) patients in NFX group, SXT group and placebo group became afebrile (body temperature $<$ 38 ^{o}C) within 48 hours after treatment (on day 2). On day 2, one of the patient in SXT group who had no fever before treatment became febrile, this is 2.0 % of the whole patients in SXT group or 3.6 % of the febrile patients (1 out of 28). On day 3, all of the patients in NFX group and SXT group became afebrile except one patient in placebo group developed fever after treatment.

Comparing the results statistically, the number of febrile patients during the first 24 hours were not statistically different in all three treatment groups

($p > 0.1$). The efficacy to alleviate the fever in all these three treatment group were not statistically different in 48 hours after treatment ($p > 0.1$).

Table 18. Results of the Fever Response

	NFX group No./total (%)	SXT group No./total (%)	placebo group No./total (%)
Took antipyretic before treatment	6/49 (12.2 %)	8/50 (16.0 %)	6/55 (10.1 %)
Took antipyretic before treatment but still febrile	3/6 (50.0 %)	2/8 (25.0 %)	3/6 (50.0 %)
Having fever before treatment started and/or having fever on day 1	26/49 (53.0 %)	22/50 (44.0 %)	26/55 (47.3 %)
Afebrile within 48 hours	25/26 (96.2 %)	22/22 (100 %)	24/26 (92.3 %)
Mean afebrile day	(1-2 days)	(1-2 days)	(1-2 days)
Those with normal temperature on admission who developed fever after treatment	0 (0.0 %)	1/28 (3.6 %)	1/29 (3.4 %)

6. Results of Other Clinical Assessments

On admission day prior to initiating of treatment white blood cells (WBC), red blood cells (RBC) and mucus found in stools were recorded as shown in table 19. The changes of mucus found in patients' stools are also shown in table 19.

Table 19. Results of WBC, RBC and Mucus

Descriptions	NFX group No./total(%)	SXT group No./total(%)	placebo group No./total(%)
WBC on day 0	28/43(65.1%)	29/45(64.4%)	37/49(75.5%)
RBC on day 0	20/43(46.5%)	16/45(35.6%)	24/49(48.9%)
Mucus on day 0	27/46(58.7%)	24/43(55.8%)	37/48(77.0%)
No mucus in stools within 48 hours	15/21(71.4%)	19/20(95.0%)	23/31(74.1%)
No mucus in stools within 72 hours	19/21(90.5%)	22/22(100%)	29/32(90.6%)

Skin turgor was also recorded and defined as normal or reduced. The changes in signs of patients'skin turgor are shown in table 20. All the patients with reduced skin turgor on admission day had normal skin turgor at 48 hours after treatment in all three groups.

Table 20. Results of the Skin Turgor

	NFX group No./total (%)	SXT group No./total (%)	placebo group No./total (%)
Reduced skin turgor before treatment started	5/49 (10.2 %)	8/50 (16.0 %)	6/55 (10.9 %)
Normal skin turgor within 48 hours	5/5 (100 %)	8/8 (100 %)	6/6 (100 %)

Note: Two patients with normal skin turgor prior to initiation of treatment in placebo group developed reduced skin turgor after treatment (one patient on day 1, another one patient on day 2)

In conclusion, at the time of admission white blood cells, red blood cells in stools and the signs of skin turgor of these three treatment groups were not statistically different ($p > 0.1$). However, the mucus found in stools of the patients in placebo group at the time of admission were statistically higher than NFX group and SXT group ($p < 0.1$ and < 0.05 respectively). It is found that the efficacy of these study drugs to improve the signs of mucus in stools were not statistically different both after 48 hours and 72 hours after treatments ($p > 0.05$ and >0.1 respectively).

7. Result of the Changes in Hematopoietic Functions

The results of the hematological test and serum creatinine determination prior to the initiation of therapy (day 0) and at 12-24 hours after the completion of the trial drugs (day P) are shown in table 21. The results on day 0 and day P were compared, using the method of paired comparasion. There were statistically difference in the values of white blood cells, neutrophils (PMN), neutrophils (Band), eosinophils , lymphocytes and serum creatinine on day 0 and day P in all three treatment groups (at $p = 0.05$). However, the changes in these values among these three treatment groups were similar to each other.

The incommon in white blood count, neutrophils count and lymphocytes count on admission were due to host response to infection. Eosinophils count was suppressed during acute infection due to endogenous steroid and returned to its normal level after the infection subsided. The decrease in the values of serum creatinine may due to the improvement in renal blood flow after the patients recover from dehydration.

Table 21. Results of the Changes in Hematopoietic Functions

BLOOD CONSTITUENT	NORMAL RANGE	NFX GROUP			SXT GROUP			PLACEBO GROUP		
		NUMBER OF PATIENTS	MEAN DAY 0	MEAN DAY P	NUMBER OF PATIENTS	MEAN DAY 0	MEAN DAY P	NUMBER OF PATIENTS	MEAN DAY 0	MEAN DAY P
Haemoglobin	MALE 14.2 - 16.4 gm%	24	14.33	14.15	30	14.98	14.41	28	15.22	14.56
	FEMALE 12.4 - 14.4 gm%	23	13.34	13.22	17	12.74	12.41	27	12.92	12.26
Haematocrit	MALE 42.9 - 49.1 %	24	41.67	41.04	32	44.00	41.97	27	44.30	42.59
	FEMALE 37.3 - 42.7 %	25	39.76	38.68	17	38.29	37.06	27	38.74	36.63
White blood cell	5,000 - 10,000 cells/mm^3	49	12,772	6,570	49	12,208	6,908	54	12,970	6,973
Neutrophils (PMN)	40 - 75 %	49	78.12	48.55	49	75.57	42.73	54	76.56	48.06
Neutrophils (Band)	3 - 5 %	49	5.63	0.88	49	7.96	1.26	54	6.85	1.35
Eosinophils	1 - 6 %	49	1.16	6.29	49	1.55	8.04	53	0.92	4.42
Basophils	less than 1%	49	0.16	0.16	48	0.08	0.33	53	0.08	0.28
Monocytes	2 - 10 %	46	2.02	2.07	46	1.82	2.17	54	1.81	1.70
Lymphocytes	20 - 50 %	49	12.94	42.53	49	12.87	45.59	54	13.80	44.35
Serum creatinine	0.5 - 1.5 mg/dl	49	1.32	1.05	48	1.71	1.15	54	1.24	1.03

8. Adverse Drug Experiences

Results of the adverse drug experiences are shown in table 22. The symptoms reported from the patients in NFX group [20 out of 49 (40.8 %)] and placebo group [18 out of 55 (32.7 %)] were higher than those in SXT group [6 out of 50 (12.0 %)]. The symptoms reported in this study included gastrointestinal symptoms, e.g. abdominal discomfort, abdominal pain, flatulence etc. and headache. All these symptoms were of mild or moderate intensity and of a transient nature. Most of them were thought to happen unlikely or possible due to the study drugs. Some of these symptoms may be related to the gastrointestinal infections or due to the other medications. None of these symptoms did require any measures to be taken, and the treatment period was complete in all cases.

Table 22. The Result of the Adverse Events

MFX GROUP / ADVERSE EVENT	PATIENT-NUMBER	START	END	SEVERITY	DUE TO DISEASE OR OTHER MEDICINE(S) (DIS OR OTHER MED)	DUE TO STUDY DRUG	OUTCOME
ABD.DISCOMFORT (BELCH)	134	DAY 2	DAY 3	MILD	DIS-OTHER MED	UNLIKELY	TOLERATE-CONT TX
ABD.DISCOMFORT	227	DAY 1	-	MILD	OTHER MED	UNLIKELY	DISAPPEAR-CONT TX
ABD.DISCOMFORT	248	DAY 0	DAY 0	MILD	DIS	UNLIKELY	DISAPPEAR-CONT TX
ABD.DISCOMFORT	2	DAY 1	DAY 2	MILD	DIS-OTHER MED	POSSIBLE	DISAPPEAR-CONT TX
ABD.DISCOMFORT	229	DAY 1	DAY 1	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX
ABD.DISCOMFORT	260	DAY 1	DAY 1	MILD	DIS	POSSIBLE	DISAPPEAR-CONT TX
ABD.DISCOMFORT	1	DAY 1	DAY 3	MODE	DIS	UNLIKELY	TOLERATE-CONT TX
ABD.PAIN	21	DAY 0	DAY 3	MILD	DIS	UNLIKELY	TOLERATE-CONT TX
ABD.PAIN	62	DAY 0	DAY 2	MILD	DIS	UNLIKELY	DISAPPEAR-CONT TX
ABD.PAIN	118	DAY 0	DAY 1	MILD	DIS	UNLIKELY	DISAPPEAR-CONT TX
FLATULENCE	123	DAY 1	DAY 3	MILD	DIS-OTHER MED	UNLIKELY	TOLERATE-CONT TX
FLATULENCE	26	DAY 2	DAY 2	MILD	DIS	POSSIBLE	DISAPPEAR-CONT TX
FLATULENCE	169	DAY 1	DAY 1	MILD	DIS-OTHER MED	POSSIBLE	DISAPPEAR-CONT TX
FLATULENCE	58	DAY 0	DAY 1	MODE	DIS	POSSIBLE	DISAPPEAR-CONT TX
HEADACHE	189	DAY 2	DAY 2	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX
HEADACHE	242	DAY 0	DAY 3	MODE	DIS	UNLIKELY	TOLERATE-CONT TX
HEADACHE	248	DAY 4	F/U	MODE	NO	UNLIKELY	APPEAR AFTER TX
HEADACHE	134	DAY 2	DAY 3	MODE	NO	POSSIBLE	TOLERATE-CONT TX
HEADACHE	192	DAY 2	DAY 2	SEVE	NO	POSSIBLE	DISAPPEAR-CONT TX
MIGRAINE	8	DAY 1	DAY 2	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX
MIGRAINE	1	DAY 2	DAY 4	MODE	NO	POSSIBLE	TOLERATE-CONT TX
MIGRAINE	123	DAY 1	DAY 1	MODE	NO	POSSIBLE	DISAPPEAR-CONT TX
MUCUS BLOODY STOOL	175	DAY 0	DAY 3	MODE	DIS	UNLIKELY	TOLERATE-CONT TX
VIRTIGO	216	DAY 2	DAY 2	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX
VOMIT	192	DAY 1	DAY 2	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX

SXT GROUP / ADVERSE EVENT	PATIENT-NUMBER	START	END	SEVERITY	DUE TO DISEASE OR OTHER MEDICINE(S) (DIS OR OTHER MED)	DUE TO STUDY DRUG	OUTCOME
ABD.DISCOMFORT	163	DAY 3	F/U	MILD	NO	UNLIKELY	APPEAR AFTER TX
ABD.DISCOMFORT	176	DAY 3	DAY 3	MILD	DIS	UNLIKELY	TOLERATE-CONT TX
ABD.DISCOMFORT	103	DAY 0	DAY 0	MODE	OTHER MED	UNLIKELY	DISAPPEAR-CONT TX
ABD.DISCOMFORT	11	DAY 1	F/U	MODE	DIS-OTHER MED	POSSIBLE	TOLERATE-CONT TX
FLATULENCE	133	DAY 1	F/U	MILD	OTHER MED	POSSIBLE	TOLERATE-CONT TX
FLATULENCE	43	DAY 1	DAY 2	MODE	NO	POSSIBLE	DISAPPEAR-CONT TX
HEADACHE	11	DAY 0	DAY 1	MODE	NO	POSSIBLE	DISAPPEAR-CONT TX

Note: ABD.DISCOMFORT = Abdominal discomfort, ABD.PAIN = Abdominal pain
ERY.RASH = Erythematous rash
F/U = Follow up day (day 10-15)
CONT TX = Continuous treatment
APPEAR AFTER TX = Appeared after treatment had been finished

Table 22. (cont.)

PLACEBO GROUP / ADVERSE EVENT	PATIENT-NUMBER	START	END	SEVERITY	DUE TO DISEASE OR OTHER MEDICINE(S) (DIS OR OTHER MED)	DUE TO STUDY DRUG	OUTCOME
ABD.DISCOMFORT (FULLNESS)	136	F/U	-	MILD	OTHER MED	UNLIKELY	APPEAR AFTER TX
ABD.DISCOMFORT	247	DAY 0	DAY 1	MILD	DIS	UNLIKELY	DISAPPEAR-CONT TX
ABD.DISCOMFORT (BELCH)	117	DAY 0	DAY 3	MILD	NO	POSSIBLE	TOLERATE-CONT TX
ABD.DISCOMFORT (BELCH)	22	DAY 3	F/U	MILD	NO	PROBABLE	APPEAR AFTER TX
ABD.DISCOMFORT (BELCH)	124	F/U	-	MODE	OTHER MED	UNLIKELY	APPEAR AFTER TX
ABD.PAIN	114	DAY 2	DAY 2	MILD	DIS	UNLIKELY	DISAPPEAR-CONT TX
ABD.PAIN	67	DAY 0	DAY 1	MILD	DIS	POSSIBLE	DISAPPEAR-CONT TX
ABD.PAIN	28	DAY 0	F/U	MODE	DIS	UNLIKELY	TOLERATE-CONT TX
ABD.PAIN	95	DAY 0	DAY 2	MODE	DIS	UNLIKELY	DISAPPEAR-CONT TX
ABD.PAIN	137	DAY 1	DAY 2	MODE	DIS	UNLIKELY	DISAPPEAR-CONT TX
ABD.PAIN	197	DAY 0	DAY 3	MODE	DIS	UNLIKELY	TOLERATE-CONT TX
ABD.PAIN	127	DAY 1	DAY 1	MODE	DIS	POSSIBLE	DISAPPEAR-CONT TX
ERY.RASH (ALL OVER)	7	DAY 1	DAY 2	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX
ERY.RASH (FACE)	22	BAY 3	DAY 3	MILD	NO	PROBABLE	DISAPPEAR-CONT TX
FLATULENCE	67	DAY 0	DAY 1	MILD	DIS	POSSIBLE	DISAPPEAR-CONT TX
FLATULENCE	29	DAY 0	DAY 1	MODE	DIS	POSSIBLE	DISAPPEAR-CONT TX
HEADACHE	114	DAY 1	DAY 2	MILD	DIS	UNLIKELY	DISAPPEAR-CONT TX
HEADACHE	188	DAY 1	DAY 2	MILD	OTHER MED	UNLIKELY	DISAPPEAR-CONT TX
HEADACHE	214	DAY 1	DAY 2	MODE	OTHER MED	UNLIKELY	DISAPPEAR-CONT TX
HEADACHE	205	DAY 1	DAY 2	MODE	NO	POSSIBLE	DISAPPEAR-CONT TX
MIGRAINE	183	DAY 1	DAY 2	MODE	DIS-OTHER MED	UNLIKELY	DISAPPEAR-CONT TX
MIGRAINE	29	DAY 0	DAY 1	MODE	NO	POSSIBLE	DISAPPEAR-CONT TX
MYALGIA	127	DAY 2	DAY 2	MILD	NO	POSSIBLE	DISAPPEAR-CONT TX
VOMIT,SEVERE DIARRHOEA	205	DAY 2	DAY 3	MODE	DIS	POSSIBLE	TOLERATE-CONT TX
WEAK	183	DAY 3	F/U	MILD	DIS	UNLIKELY	APPEAR AFTER TX

Note: ABD.DISCOMFORT = Abdominal discomfort, ABD.PAIN = Abdominal pain
ERY.RASH = Erythematous rash
F/U = Follow up day (day 10-15)
CONT TX = Continuous treatment
APPEAR AFTER TX = Appeared after treatment had been finished