Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2019, 11(4):345-351. DOI: 10.15835/nsb11410491
Original Article

Evaluation of Leukocyte Mobilization and Platelet Aggregatory Effects of Ciprofloxacin, Lincomycin and Erythromycin in Wistar Albino Rats

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Abstract

This study evaluated leucocyte mobilization and platelet aggregation effects of ciprofloxacin, lincomycin and Erythromycin in Wistar albino rats. Thirty-three female Wistar albino rats weighing 130-160 g and three male Wistar albino rats weighing 188-194 g, fed commercial growers' mash and clean tap water were used. In leukocytes assay, thirty-three adult female Wistar rats were assigned into five treatments, eleven groups of three rats per group. First three groups were treated 10 mg kg^{-1}, 20 mg kg^{-1} and 40 mg kg^{-1} of ciprofloxacin, next three groups same doses of lincomycin, next three groups same doses of Erythromycin, the tenth group received Indomethacin 5 mg kg^{-1} (reference drug), the last group 5 mg kg^{-1} normal saline. Assay on platelet aggregator activity involved collection of blood samples (10 ml each, 1% EDTA, centrifuged at 3000 rpm for 10 minutes), test drugs dissolved in 1 mg/ml distilled water and Indomethacin (the reference drug). The three antibiotics significantly reduced leucocyte mobilization into the affected tissue. They all had their maximum inhibitory effects at the highest dose (40 mg kg^{-1}) compared with indomethacin. Erythromycin 40 mg kg^{-1} showed the highest inhibitory effect on leucocyte migration. Whereas ciprofloxacin and erythromycin had stepwise increase in absorbance from time 0 secs through to time 120 secs, lincomycin showed a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. The testing drugs prevented leukocyte mobilization also had stepwise increase in absorbance from time 0 secs through to time 120 secs in platelet aggregatory activity assay to some extent.

Keywords: ciprofloxacin; erythromycin; lincomycin; leukocyte; platelet aggregatory; rats

Introduction

Functionally, the immune system is protected by leukocytes. Leukocyte mobilization is part of the physiological activities that occur during inflammation. Inflammation is a protective response that involves immune cells, blood vessels, and a host of molecular mediators. Its purpose is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. Migration of leukocytes to sites of injury or inflammation is a crucial component of both innate and adaptive immunity. Such a mechanism could clearly contribute to the ability of the immune system to mount an optimum, appropriate and localised tissue response to a wide range of extravascular stimuli without causing any untoward damage to the vasculature.

Platelets are subcellular fragments, which circulate in blood and have well established roles in thrombosis and haemostasis in adults. Platelet activation and aggregation leads to thrombotic events. Aggregation of platelets involves platelet-to-platelet adhesion, and is necessary for effective haemostasis following the initial adhesion of platelets to the site of injury (Varga-Szabo *et al*., 2009). The inhibition of platelet functions leads to bleeding disease, while over activation of platelets contributes to thrombotic diseases (Camilleri *et al*., 2011; Kei *et al*., 2011). Disrupted haemorrhage is caused by the imbalance of coagulation and anticoagulation system (Li *et al*., 2010; Bollati *et al*., 2011).

Currently, the use of antibiotics in inflammation treatment is uncommon. The dearth of literature in evaluation of leukocyte mobilization and platelet aggregator effects of ciprofloxacin, lincomycin and erythromycin in Wistar albino rats motivated the present study. This study

Received: 27 Apr 2019. Received in revised form: 10 Dec 2019. Accepted: 13 Dec 2019. Published online: 24 Dec 2019.

was specifically designed to assay for the effect of the three antibiotics (ciprofloxacin, lincomycin and erythromycin) on agar-induced leucocyte mobilization to the peritoneal cavity and platelet aggregation.

Materials and Methods

Drugs used

Ciprofloxacin (Ciprotab) used were ciprofloxacin hydrochloride U.S.P. (ciprofloxacin hydrochloride, 500 mg) soft gelatin coated tablets. They were manufactured by Geltec Pvt. Ltd., Karnataka, India. They served as a test drug. Lincomycin used was Lincomycin 500 mg capsules. They were manufactured by Mekophar (Ho Chi Minh City, Vietnam) and used as a test drug. Erythromycins used were erythromycin stearate (erythromycin base, 500 mg) film coated tablets. They were manufactured by Mekophar (Ho Chi Minh City, Vietnam). They served as a test drug. Indomethacin used was indomethacin 25 mg capsules. They as a reference drug for leukocyte mobilization and anti-platelet aggregation assays.

Nutrient agar: Prepared by dissolving 28.0 g of nutrient agar in 1000 ml of distilled water. The mixture was homogenized in a water bath at 100 °C for 10 minutes; 5 ml was dispensed into Petri dishes and autoclaved at 121 °C for 15 minutes. It was used for the first culture of *Aspergillus niger*.

Procurement and care of animal

Thirty-three female Wistar albino rats (with no history of prior use in any investigation) weighing 130 - 160 g and three male Wistar albino rats weighing 188 - 194 g were obtained from the Genetics and Experimental Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The ethical conditions and experimental protocol governing the use and conduct of experiments with live animals were strictly observed as approved by the University of Nigeria, Nsukka Senate Committee on Medical and Research ethics. The animals were hygienically housed in stainless wire rat cages furnished with drinkers and faecal gathering trays, fed with commercial growers' mash (Vital Feeds, Grand Cereals Limited, Jos, Nigeria) and clean tap water. The animals were allowed to acclimatize for two weeks before being used for the experiment. The light cycle was controlled (12 hr day/night cycle), and the room temperature was approximately 37 °C. The fungal organisms used were strains of *Aspergillus niger* which were cultured in the Department of Microbiology, University of Nigeria, Nsukka.

Collection of blood sample

About 5 ml of the blood samples was collected from each of the anaesthetized rats using the ocular puncture method described by Hoff (2000).

Effect of antibiotic on leukocyte mobilization

The effect of the test antibiotics on *in vivo* leukocyte mobilization induced by an inflammatory stimulus was investigated using the method described by Ribeiro *et al*. (1991). The study involved thirty-three adult female Wistar rats assigned into five treatments, grouped into eleven of three rats per group. First three groups were treated 10 mg kg^{-1}, 20 mg kg^{-1} and 40 mg kg^{-1} of ciprofloxacin, next three groups same doses of lincomycin, next three groups same doses of Erythromycin, the tenth group received the reference drug, Indomethacin 5 mg kg^{-1} while the last group 5 mg kg^{-1} normal saline. One hour after oral administration of the drugs, each rat in the various groups received intraperitoneal injections of 0.5 ml of 3% w/v agar suspension in distilled water. Four hours later, the rats were sacrificed and the peritoneum was washed with 5 ml of 5% solution of EDTA in phosphate buffered saline (PBS). The peritoneal fluid was recovered. Total leukocyte count (TLC) and differential leukocyte count (DLC) were performed on the peritoneal fluid.

Assay of platelet aggregatory activity

The assay used is a modification of the method of Born and Cross (1963). Various blood samples were taken from 3 adult Wistar albino rats with the aid of a capillary tube. Fresh blood (10 ml each) was drawn into plastic tubes containing 1% EDTA droplets as an anticoagulant. The tubes were centrifuged at 3000 rpm for 10 minutes. The plasma supernatant was collected, diluted twice with normal saline and used as the platelet rich plasma (PRP). The test drugs were dissolved 1 mg/ml in distilled water. Platelet rich plasma (0.2 ml), 0.4 ml of 1.47% $CaCl_2$ and varying concentrations of normal saline and the drug were incubated at room temperature for 2 min. Indomethacin was used as the reference drug. $CaCl_2$ was used to start the reaction. The absorbance of the solutions was measured at 520 nm. Changes in the absorbance were taken at zero seconds, 30 sec, 60 sec, 90 sec and 120 sec after adding $CaCl_2$.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out on the data using the IBM Statistics UK (version 20.0). The means were separated using Duncan's new multiple range test while differences in the means were considered significant at probability values less than 5% ($p < 0.05$). The results were presented as mean ± SD.

Results

Effect of ciprofloxacin on agar-induced leukocyte mobilization

Table 1 shows the effect of ciprofloxacin on agar-induced leukocyte migration in rats. There was a decrease in agar-induced mobilization of leukocytes upon treatment with ciprofloxacin. This decrease was dose dependent with Ciprofloxacin 10 mg kg^{-1}, 20 mg kg^{-1} and 40 mg kg^{-1} having total leucocyte counts of 2566.67, 2366.67 and 2000.00 respectively. Neutrophils were the most mobilized leucocytes. Basophils were barely mobilized. Indomethacin decreased leukocyte mobilization into the cavity.

Effect of lincomycin on agar-induced leukocyte mobilization

Table 2 shows the effect of lincomycin on agar-induced leukocyte migration in rats.

There was a decrease in agar-induced mobilization of leukocytes upon treatment with ciprofloxacin. This decrease was dose dependent with lincomycin 10 mg kg^{-1}, 20 mg kg^{-1} and 40 mg kg^{-1} having total leucocyte counts of 2633.33, 2266.67 and 1900.00 respectively. Neutrophils were the most mobilized leucocytes. Basophils were barely mobilized. The total cell count was lower in indomethacin when compared to all doses of the antibiotic but the difference between indomethacin and the highest dose of the drug was non-significant.

Effect of Erythromycin on agar-induced leukocyte mobilization

Table 3 shows the effect of erythromycin on agar-induced leukocyte migration in rats. There was a decrease in agar-induced mobilization of leukocytes upon treatment with ciprofloxacin. This decrease was not dose dependent but the highest concentration of the antibiotic had the least total leucocyte count. Erythromycin 10 mg kg^{-1}, 20 mg kg^{-1} and 40 mg kg^{-1} had total leucocyte counts of 2333.33, 2733.33 and 1866.67 respectively. Neutrophils were the most mobilized leucocytes. Basophils were barely mobilized. The total cell count was lower in the group treated with indomethacin when compared to the groups treated with 10 mg kg^{-1} and 20 mg kg^{-1} erythromycin but there was no difference between indomethacin and the highest dose of the drug.

Table 1. Effect of Ciprofloxacin on agar-induced leukocyte mobilization

Group	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Cipro 10 mg kg^{-1}	2566.67±57.74	59.33±10.07	36.67±10.50	3.00±1.00	2.00±1.00	0.00±0.00
Cipro 20 mg kg^{-1}	2366.67±351.3	62.00±2.00	32.00±3.61	3.67±1.53	2.33±1.53	0.00±0.00
Cipro 40 mg kg^{-1}	2000.00±100.0	59.00±4.58	36.33±7.02	2.33±2.31	2.33±0.58	0.00±0.00
Indo 5 mg kg^{-1}	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n=3; Results expressed as Mean ± SD

Table 2. Effect of Lincomycin on agar-induced leukocyte mobilization

Group	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Linco 10 mg kg^{-1}	2633.33±152.8	58.67±9.02	37.33±9.02	3.00±1.00	1.67±0.58	0.00±0.00
Linco 20 mg kg^{-1}	2266.67±115.47	57.33±6.11	38.33±5.69	2.67±0.58	1.67±0.58	0.00±0.00
Linco 40 mg kg^{-1}	1900.00±173.20	59.67±2.52	37.67±2.52	1.67±0.58	1.00±1.00	0.00±0.00
Indo 5 mg kg^{-1}	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n=3; Results expressed as Mean ± SD

Table 3. Effect of Erythromycin on agar-induced leukocyte mobilization

Group	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Erythro 10 mg kg^{-1}	2333.33±142.91	54.67±7.02	41.33±7.02	2.33±0.58	1.67±0.58	0.00±0.00
Erythro 20 mg kg^{-1}	2733.33±208.17	57.33±1.53	37.67±1.53	3.33±0.58	1.67±0.58	0.00±0.00
Erythro 40 mg kg^{-1}	1866.67±208.17	54.00±2.00	42.67±3.21	2.00±1.00	1.33±0.58	0.00±0.00
Indo 5 mg kg^{-1}	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n=3; Results expressed as Mean ± SD

Effect of the various antibiotics on agar-induced leukocyte mobilization

Table 4 is a comparative table showing the effects of the various antibiotics on leucocyte mobilization and how they compare to one another. All the antibiotics significantly reduced leucocyte mobilization into the affected tissue. They all had their maximum inhibitory effects at the highest dose (40 mg kg^{-1}) and these compared well with indomethacin. Erythromycin 40 mg kg^{-1} showed the highest inhibitory effect on leucocyte migration. The control had the highest total leucocyte count (3666.67) and the highest percentage of neutrophils that migrated (63.67%).

Effect of ciprofloxacin on platelet aggregation

Table 5 shows the effect of the antibiotic, ciprofloxacin, on platelet aggregation. The absorbance of the control was significantly lower than the absorbance of the varying concentrations of ciprofloxacin. The absorbance of samples with ciprofloxacin was higher at 0 seconds than at 120 seconds. There was a stepwise decrease in the absorbance from 0 seconds through to 120 seconds. This was the same for all concentrations of the samples with ciprofloxacin and the normal control. Indomethacin showed a different pattern. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. This was observed at all concentrations of the reference drug. The control without $CaCl_2$ had a very high absorbance when compared to the normal control samples, samples with ciprofloxacin and samples with indomethacin.

Table 4. Effect of the various antibiotics on agar-induced leukocyte mobilization

Group	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Cipro 10 mg kg^{-1}	2566.67±57.74	59.33±10.07	36.67±10.50	3.00±1.00	2.00±1.00	0.00±0.00
Cipro 20 mg kg^{-1}	2366.67±351.3	62.00±2.00	32.00±3.61	3.67±1.53	2.33±1.53	0.00±0.00
Cipro 40 mg kg^{-1}	2000.00±100.0	59.00±4.58	36.33±7.02	2.33±2.31	2.33±0.58	0.00±0.00
Linco 10 mg kg^{-1}	2633.33±152.8	58.67±9.02	37.33±9.02	3.00±1.00	1.67±0.58	0.00±0.00
Linco 20 mg kg^{-1}	2266.67±115.47	57.33±6.11	38.33±5.69	2.67±0.58	1.67±0.58	0.00±0.00
Linco 40 mg kg^{-1}	1900.00±173.20	59.67±2.52	37.67±2.52	1.67±0.58	1.00±1.00	0.00±0.00
Erythro 10 mg kg^{-1}	2333.33±142.91	54.67±7.02	41.33±7.02	2.33±0.58	1.67±0.58	0.00±0.00
Erythro 20 mg kg^{-1}	2733.33±208.17	57.33±1.53	37.67±1.53	3.33±0.58	1.67±0.58	0.00±0.00
Erythro 40 mg kg^{-1}	1866.67±208.17	54.00±2.00	42.67±3.21	2.00±1.00	1.33±0.58	0.00±0.00
Indo 5 mg kg^{-1}	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n=3; Results expressed as Mean ± SD

Table 5. Effect of Ciprofloxacin on platelet aggregation

Group	Drug (ml)	Normal saline (ml)	Absorbance (520 nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.3217±0.048	0.3190±0.046	0.3173±0.045	0.3140±0.045	0.3130±0.044
Cipro	0.1	1.7	0.3390±0.031	0.3300±0.029	0.3190±0.020	0.3100±0.017	0.3040±0.015
	0.2	1.6	0.3290±0.006	0.3300±0.009	0.3170±0.006	0.3090±0.009	0.3030±0.013
	0.4	1.4	0.3330±0.005	0.3280±0.007	0.3160±0.005	0.3050±0.004	0.3000±0.007
	0.6	1.2	0.3273±0.010	0.3240±0.005	0.3110±0.006	0.3020±0.012	0.2980±0.003
	0.8	1.0	0.3333±0.006	0.3210±0.002	0.3080±0.010	0.3000±0.007	0.2940±0.005
Indo	0.2	1.6	0.5437±0.006	0.5220±0.004	0.5317±0.009	0.5497±0.004	0.5607±0.002
	0.4	1.4	0.5230±0.009	0.5113±0.010	0.6060±0.006	0.6070±0.005	0.6140±0.009
	0.6	1.2	0.5490±0.003	0.5273±0.006	0.5840±0.018	0.5980±0.014	0.6060±0.010
Control (no $CaCl_2$)		1.8	0.4897±0.010	0.4920±0.007	0.4860±0.003	0.4880±0.009	0.4890±0.006

n=3; Results expressed as Mean ± SD

Effect of Erythromycin on platelet aggregation

Table 6 shows the effect of the antibiotic, erythromycin, on platelet aggregation. The absorbance of the control was significantly lower than the absorbance of the varying concentrations of erythromycin. The absorbance of samples with lincomycin was higher at 0 seconds than at 120 seconds. There was a stepwise decrease in the absorbance from 0 seconds through to 120 seconds. This was the same for all concentrations of the samples with erythromycin and the normal control. Indomethacin showed a different pattern. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. This was observed at all concentrations of the reference drug. The control without $CaCl_2$ had a very high absorbance when compared to the normal control samples, samples with erythromycin and samples with indomethacin.

Effect of lincomycin on platelet aggregation

Table 7 shows the effect of the antibiotic, lincomycin, on platelet aggregation. The absorbance of the control was significantly lower than the absorbance of the varying concentrations of lincomycin. The absorbance of samples with erythromycin was lower at 0 seconds than at 120 seconds. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. Indomethacin showed a similar pattern. The control without $CaCl_2$ had a very high absorbance when compared to the normal control samples, samples with lincomycin and samples with indomethacin.

Effect of the various antibiotics on platelet aggregation

Table 8 shows the effect of the various antibiotics on platelet aggregation. Ciprofloxacin, erythromycin and the normal control all followed a similar trend. They all had stepwise increase in absorbance from time 0 secs through to time 120 secs. The pattern in lincomycin differed from the other two drugs. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds and this was similar to what was obtained from indomethacin.

Table 6. Effect of Erythromycin on platelet aggregation

Group	Drug (ml)	Normal saline (ml)	Absorbance (520 nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.3217±0.048	0.3190±0.046	0.3173±0.045	0.3140±0.045	0.3130±0.044
Erythro	0.1	1.7	0.3310±0.010	0.3240±0.006	0.3153±0.003	0.3090±0.004	0.3000±0.011
	0.2	1.6	0.3280±0.006	0.3200±0.003	0.3140±0.002	0.3090±0.004	0.3000±0.006
	0.4	1.4	0.3257±0.005	0.3190±0.002	0.3110±0.003	0.3050±0.007	0.3010±0.008
	0.6	1.2	0.3217±0.007	0.3140±0.004	0.3120±0.002	0.3020±0.008	0.2940±0.005
	0.8	1.0	0.3193±0.006	0.3110±0.003	0.3050±0.005	0.3000±0.005	0.2940±0.003
Indo	0.2	1.6	0.5437±0.006	0.5220±0.004	0.5317±0.009	0.5497±0.004	0.5607±0.002
	0.4	1.4	0.5230±0.009	0.5113±0.010	0.6060±0.006	0.6070±0.005	0.6140±0.009
	0.6	1.2	0.5490±0.003	0.5273±0.006	0.5840±0.018	0.5980±0.014	0.6060±0.010
Control (no CaCl2)		1.8	0.4897±0.010	0.4920±0.007	0.4860±0.003	0.4880±0.009	0.4890±0.006

n=3; Results expressed as Mean ± SD

Table 7. Effect of Lincomycin on platelet aggregation

Group	Drug (ml)	Normal saline (ml)	Absorbance (520 nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.3217±0.048	0.3190±0.046	0.3173±0.045	0.3140±0.045	0.3130±0.044
Linco	0.1	1.7	0.3130±0.005	0.3111±0.006	0.3223±0.003	0.3340±0.005	0.3500±0.002
	0.2	1.6	0.3100±0.015	0.3126±0.017	0.3230±0.020	0.3390±0.005	0.3440±0.002
	0.4	1.4	0.3200±0.005	0.3139±0.012	0.3293±0.017	0.3400±0.004	0.3530±0.004
	0.6	1.2	0.3407±0.005	0.3151±0.023	0.3510±0.020	0.3660±0.006	0.3653±0.003
	0.8	1.0	0.3333±0.018	0.3167±0.018	0.3570±0.005	0.3570±0.009	0.3570±0.002
Indo	0.2	1.6	0.5437±0.006	0.5220±0.004	0.5317±0.009	0.5497±0.004	0.5607±0.002
	0.4	1.4	0.5230±0.009	0.5113±0.010	0.6060±0.006	0.6070±0.005	0.6140±0.009
	0.6	1.2	0.5490±0.003	0.5273±0.006	0.5840±0.018	0.5980±0.014	0.6060±0.010
Control (no CaCl2)		1.8	0.4897±0.010	0.4920±0.007	0.4860±0.003	0.4880±0.009	0.4890±0.006

n=3; Results expressed as Mean ± SD

Table 8. Effect of the various antibiotics on platelet aggregation (comparative)

Group	Drug (ml)	Normal saline (ml)	Absorbance (520 nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.3217±0.048	0.3190±0.046	0.3173±0.045	0.3140±0.045	0.3130±0.044
Cipro	0.1	1.7	0.3390±0.031	0.3300±0.029	0.3190±0.020	0.3100±0.017	0.3040±0.015
	0.2	1.6	0.3290±0.006	0.3300±0.009	0.3170±0.006	0.3090±0.009	0.3030±0.013
	0.4	1.4	0.3330±0.005	0.3280±0.007	0.3160±0.005	0.3050±0.004	0.3000±0.007
	0.6	1.2	0.3273±0.010	0.3240±0.005	0.3110±0.006	0.3020±0.012	0.2980±0.003
	0.8	1.0	0.3333±0.006	0.3210±0.002	0.3080±0.010	0.3000±0.007	0.2940±0.005
Erythro	0.1	1.7	0.3310±0.010	0.3240±0.006	0.3153±0.003	0.3090±0.004	0.3000±0.011
	0.2	1.6	0.3280±0.006	0.3200±0.003	0.3140±0.002	0.3090±0.004	0.3000±0.006
	0.4	1.4	0.3257±0.005	0.3190±0.002	0.3110±0.003	0.3050±0.007	0.3010±0.008
	0.6	1.2	0.3217±0.007	0.3140±0.004	0.3120±0.002	0.3020±0.008	0.2940±0.005
	0.8	1.0	0.3193±0.006	0.3110±0.003	0.3050±0.005	0.3000±0.005	0.2940±0.003
Linco	0.1	1.7	0.3130±0.005	0.3111±0.006	0.3223±0.003	0.3340±0.005	0.3500±0.002
	0.2	1.6	0.3100±0.015	0.3126±0.017	0.3230±0.020	0.3390±0.005	0.3440±0.002
	0.4	1.4	0.3200±0.005	0.3139±0.012	0.3293±0.017	0.3400±0.004	0.3530±0.004
	0.6	1.2	0.3407±0.005	0.3151±0.023	0.3510±0.020	0.3660±0.006	0.3653±0.003
	0.8	1.0	0.3333±0.018	0.3167±0.018	0.3570±0.005	0.3570±0.009	0.3570±0.002
Indo	0.2	1.6	0.5437±0.006	0.5220±0.004	0.5317±0.009	0.5497±0.004	0.5607±0.002
	0.4	1.4	0.5230±0.009	0.5113±0.010	0.6060±0.006	0.6070±0.005	0.6140±0.009
	0.6	1.2	0.5490±0.003	0.5273±0.006	0.5840±0.018	0.5980±0.014	0.6060±0.010
Control (no CaCl2)		1.8	0.4897±0.010	0.4920±0.007	0.4860±0.003	0.4880±0.009	0.4890±0.006

n=3; Results expressed as Mean ± SD

Discussion

Evaluation of platelet aggregatory and leukocyte mobilization activities of ciprofloxacin, lincomycin and erythromycin in Wistar albino rats (*in vivo* and *in vitro)* was the aim of the present study.

There was dose dependent decrease in leucocyte mobilization upon treatment with all three antibiotics. The agar suspension was able to cause an injury which was responded to by the drugs, probably suppressing the proliferation of inflammatory mediators that could cause an increase in the production of leucocytes that could migrate to the area. Of the various leucocytes mobilized, neutrophils were the most abundant. The numbers of neutrophils and other phagocytic cells increase during injury and are directly responsible for the high number of leukocytes that are mobilized to the site of an injury (Vega and Corbi, 2006). These neutrophils attack and decimate the cause of infection using lactoferrin, gelatinase and myeloperoxidase present in granules. They degrade the extracellular matrix, digest the phagocytosed material and bring about inflammation (Dale *et al*., 2008). Indomethacin used in this study decreased the mobilization of leukocytes and this is in line with Bhattacherjee *et al*. (1983) that stated that high doses of indomethacin inhibited the accumulation of leukocytes.

In the platelet aggregation test, $CaCl_2$ caused an increase in calcium concentration in the test-tube. This would cause a number of .structural and functional changes of the platelet in the platelet rich plasma (PRP). The increased calcium concentration could also stimulate phospholipase A_2 to metabolize thromboxane A_2 which is a potent activator of platelets. The combined effect brings about shape changes which allow the platelets to interact with one another to form aggregates. As platelets aggregate, the light transmission of the sample increases hence the absorbance decreases. Ciprofloxacin and erythromycin showed a stepwise decrease in absorbance from time zero sec through to time 120 sec when $CaCl_2$ was added. This showed that both drugs had no effect on platelet aggregation. Lincomycin caused a decrease in absorbance when $CaCl_2$ was added but showed a sharp increase from around 30 seconds through to 120 seconds. This trend was similar to that of indomethacin. This showed that the addition of $CaCl_2$ caused the platelets to aggregate but the presence of the drug, lincomycin, stopped this and possibly prevented further shape change and aggregation hence the increase in absorbance.

Conclusions

This study has demonstrated that these agents can exert other therapeutic effects independent of their antibacterial activity. Lincomycin had more activity on platelet aggregation tests. Erythromycin inhibited paw oedema especially at the second stage. All three prevented leukocyte mobilization. Despite the fact that there were non-significant differences between the maximum effects of the test drugs and the reference drugs, these drugs actually have these effects to an extent.

Acknowledgements

Our sincerely appreciation goes to Mr and Mrs Eugine Chukwunelo for financing this research.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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