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# *IN-VIVO* STUDIES ON THE BIOEQUIVALENCE OF SOME BRANDS OF OFLOXACIN AND LEVO-FLOXACIN TABLETS MARKETED IN NIGERIA

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*ABSTRACT*

***Objective*** *This study was aimed to assess the bioequivalence of randomly selected brands of ofloxacin and its levo-isomer, levo-floxacin tablets marketed in Nigeria.*

***Method*** *Bioavailability assessment was conducted by measuring the concentration of drugs in the urine and bioavailability data was presented as cumulative quantity of drugs recovered in urine in 36 hours. Microbiological assay technique was used to analyze urine samples.*

***Main Outcome Measure*** *Cumulative quantity of drugs recovered in urine in 36 hours and minimum inhibitory concentrations.*

***Result*** *The five brands of ofloxacin and the three brands of levofloxacin showed the same minimum inhibitory concentration value against the test strain of Staphylococcus aureus of 0.483µg/ml and 1.953µg/ml respectively. The result of the percentage cumulative quantity of drug recovered from urine showed that there was no statistical significant difference among ofloxacin and levofloxacin brands.*

***Conclusion*** *The various brands of ofloxacin ($X_1 – X_5$) and levofloxacin ($Y_1 – Y_3$) in Nigeria exhibited same bioavailability data in vivo and can be said to be bioequivalents.*

***Keywords*** *Antibiotics, Bioequivalence, Generic substitution, Microbiological assay, Nigeria*

## INTRODUCTION

Generic substitution is defined as dispensing of product that is generically equivalent to the prescribed product with the same active ingredients in the same dosage form, and identical in strength, concentration, and route of administration [1]. The indication for the use of generic names for drug purchasing as well as prescribing is precisely to facilitate drug substitution whenever appropriate. It has been stated that the use of generic names for drug purchasing and prescribing carries considerations of clarity, quality, and price [1] and it forms one of the core drug use indicator for the assessment of rational prescribing behaviour in practice settings [2].

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Nevertheless, opponents of generic substitution argue that the quality of generic drugs might be inferior to that of brand-name products [1]. It is therefore important to ensure that generics substitutes are bio-equivalent. This is particularly important for developing countries like Nigeria where drug distribution and supply is known to be erratic. Factors that often necessitate the need for adequate bioequivalence studies include treatment failures, high cost of patented products, increase in resistance strains and sub-standardization. Bioequivalence studies will ensure that the characteristics of finished product conform to the appropriate standard specifications in terms of product identity, purity, potency, bioavailability, pharmacokinetics and therapeutic activity [3]. For these reasons, regulatory authorities, which have to make decisions about the licensing of products that are generic equivalents of patented products, lay importance on evidence of bioequivalence, i.e. evidence that the new product behaves sufficiently similarly to the existing one to be substituted for it without causing clinical problems [3].

Bioequivalence studies are conducted employing bioavailability parameters. Bioavailability determines the rate and extent of absorption of a drug from its dosage form as determined by its concentration time curve in a systemic circulation or by its excretion in urine [4, 5]. The relative bioavailability of a drug is determined by comparing the blood level and/or urinary excretion after administration of the test form of the drug and a reference form (an innovator) in the same formulation type utilizing the same route of administration [6, 4]. Usually, an innovator product is that which was first authorized (as a patented drug) on the basis of documentation of safety, quality and efficacy [7]. Therefore, two pharmaceutically equivalent drug products are considered to be bioequivalent when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable test condition [8, 9]. There are *in vitro* and *in vivo* tests that can be utilized to estimate the bioequivalence of drug product, but only the *in vivo* tests are employed in this study.

This study was aimed to assess the bioequivalence of randomly selected brands of ofloxacin and its levo-isomer, levo-floxacin tablets marketed in Nigeria..

## METHODS

Five commercial brands of ofloxacin 200 mg ($X_1 – Y_5$) and 3 commercial brands of levo-floxacin 500 mg ($Y_1 – Y_3$) were obtained randomly from different pharmacies and patent medicines stores in Nigeria. Ten healthy adult male volunteers between the ages of 19 – 35, weighing between 72 to 90 kg body-weight were selected for the study. Federal Medical Center, Abakaliki, Ebonyi State, Nigeria granted ethical approval for the study. Informed consent was obtained from each subject. Prior to initiation of the study the participants were subjected to thorough physical examination and their medical history taken. Basic tests like liver function test (LFT), urinalysis, full blood count (FBC) and blood sugar levels were conducted for each subject, to certify that they were medically fit for the study. The subjects were not

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permitted to take any drug two weeks before trials and during the trials.

The experimental design employed to determine comparative bioavailability (bioequivalence) of the drug brands was the Latin square crossover design [10]. Fifteen subjects were randomly assigned to five different groups (three per group) ensuring that uniformity existed between groups with respect of age, body weight and sex. Each group received a particular treatment or brand the treatment were separated by a 7-day washout period and the design was balanced over weeks. The volunteers were fasted overnight prior to and 4 hours immediately after administration of a 200 mg ofloxacin tablet and 500 mg levofloxacin tablets. No beverage such as coffee, milk or diet drink was permitted during the fasting period. Alcoholic drink was also restricted. Each of the tablet brands was administered with 300 ml of water. An additional 100 ml of water was given each hour for the first three hours after dosing. The subjects were ambulatory for the first twenty-four hours of treatment, and for the remaining 12 hours were permitted to proceed with their normal daily routine in so far as possible, but they were not permitted to engage in any strenuous or athletic activities during the period of the study.

Total urine voids were collected at intervals of 0 – 4, 4 – 8, 8 – 12, 12 – 16, 15 – 20, 20 – 24, 30 – 36 hours post administration. The total volume of each interval was recorded and a 10 ml sample each was frozen until assay. Spectrophotometric method was employed to determine the cumulative quantities excreted in urine and the mean excreted amount of drug was computed.

The microbiological assay technique was used for the analysis of urine samples. Molten Mueller-Hinton agar (MHA at 56$^{o}$C) seeded with a standardized inoculum 0.5 MacFarland standard of a clinical strain of *Staphyloccus aureus* [11] was allowed to solidify. Thereafter, 5 mm holes were bored on each MHA using a sterile cork borer. Various concentrations of a standard solution of ofloxacin and levofloxacin (31.25 – 1000 µg/ml) and the various urine samples obtained from each subject at different intervals were randomly introduced into different holes (40 µl per hole). After allowing for 30 minutes pre-diffusion at room temperature, the plates were then incubated at 37$^{o}$C for 24 hours. The inhibition zone diameters (IZDs) [12] were measured. The IZDs of the standard was used to construct a dose-response plot from which the concentration of ofloxacin and levofloxacin in each urine sample was calculated by fitting their respective IZDs into a regression equation derived from the standard dose-response plot.Statistical analyses were carried out using SPSS for Windows (version 14; SPSS, Chicago, IL). Data were summarized as mean ± SD. Group comparison was conducted using ANOVA. Sub-group analysis was carried out using the Post hoc test, LSD. A two-tailed significance level of 0.05 was used.

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**Table 1: Latin square crossover design treatment for ofloxacin and levofloxacin brands**

| ***Ofloxacin*** | | | | | | |
|---|---|---|---|---|---|---|
| *Group* | *Subject* | *Week 1* | *Week 2* | *Week 3* | *Week 4* | *Week 5* |
| 1 | 1 | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
| | 2 | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
| | 3 | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
| 2 | 4 | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_1$ |
| | 5 | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_1$ |
| | 6 | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_1$ |
| 3 | 7 | $X_3$ | $X_4$ | $X_5$ | $X_1$ | $X_2$ |
| | 8 | $X_3$ | $X_4$ | $X_5$ | $X_1$ | $X_2$ |
| | 9 | $X_3$ | $X_4$ | $X_5$ | $X_1$ | $X_2$ |
| 4 | 10 | $X_4$ | $X_5$ | $X_1$ | $X_2$ | $X_3$ |
| | 11 | $X_4$ | $X_5$ | $X_1$ | $X_2$ | $X_3$ |
| | 12 | $X_4$ | $X_5$ | $X_1$ | $X_2$ | $X_3$ |
| 5 | 13 | $X_5$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ |
| | 14 | $X_5$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ |
| | 15 | $X_5$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ |

| ***Levofloxacin*** | | | | |
|---|---|---|---|---|
| *Group* | *Subject* | *Week 1* | *Week 2* | *Week 3* |
| 1 | 1 | $Y_1$ | $Y_2$ | $Y_3$ |
| | 2 | $Y_1$ | $Y_2$ | $Y_3$ |
| | 3 | $Y_1$ | $Y_2$ | $Y_3$ |
| 2 | 4 | $Y_2$ | $Y_3$ | $Y_1$ |
| | 5 | $Y_2$ | $Y_3$ | $Y_1$ |
| | 6 | $Y_2$ | $Y_3$ | $Y_1$ |
| 3 | 7 | $Y_3$ | $Y_1$ | $Y_2$ |
| | 8 | $Y_3$ | $Y_1$ | $Y_2$ |
| | 9 | $Y_3$ | $Y_1$ | |
| 4 | 10 | $Y_1$ | $Y_2$ | $Y_3$ |
| | 11 | $Y_1$ | $Y_2$ | $Y_3$ |
| | 12 | $Y_1$ | $Y_2$ | $Y_3$ |
| 5 | 13 | $Y_2$ | $Y_3$ | $Y_1$ |
| | 14 | $Y_2$ | $Y_3$ | $Y_1$ |
| | 15 | $Y_2$ | $Y_3$ | $Y_1$ |

**Table 2: Minimum Inhibitory Concentration (MIC) and Cumulative quantity of drugs recovered in urine in 36 hours**

| ***Brands*** | ***Mean cumulative quantity excreted in 36 hrs (mg)*** | ***Mean Recovery (%)*** | ***Mean maximum excretion rate (mg/hr)*** | ***MIC (µg/ml)*** |
|---|---|---|---|---|
| $X_1$ | 20.80 ± 0.74 | 10.40 | 2.14 ± 0.73 | 0.488 |
| $X_2$ | 20.52 ± 0.55 | 10.26 | 1.75 ± 0.20 | 0.488 |

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| | | | | |
|---|---|---|---|---|
| $X_3$ | 20.83 ± 0.50 | 10.42 | 2.09 ± 0.36 | 0.488 |
| $X_4$ | 20.64 ± 0.75 | 10.32 | 1.86 ± 0.18 | 0.488 |
| $X_5$ | 20.62 ± 0.41 | 10.31 | 1.73 ± 0.27 | 0.488 |
| $Y_1$ | 148.41 ± 5.41 | 29.69 | 12.15 ± 3.59 | 1.953 |
| $Y_2$ | 146.58± 8.36 | 29.32 | 11.47 ± 3.00 | 1.953 |
| $Y_3$ | 147.50 ± 4.85 | 28.82 | 10.76 ± 3.15 | 1.953 |

n = 15; time for maximum excretion rate = 6 hours

## RESULTS

There were many brands of ofloxacin and levofloxacin tablets in circulation in the country. They were of varying shapes, sizes and colours. Ofloxacin prize range varies from $3.3 – $7.3 per pack of 10 tablets while levofloxacin varies from $4 – $30. The cheaper brands were mostly imported from Asian countries. Cumulative quantity excreted in urine was directly related to the total amount of drug absorbed and indicates the extent of absorption. The percentage recovery indicated that brand $Y_1$ was the brand with the highest recovery within 36 hrs of 29.69%, while the least is $X_2$ with 10.26%. The five brands of ofloxacin and the three brands of levofloxacin showed the same minimum inhibitory concentration (MIC) value against the test strain of *Staphylococcus aureus* of 0.483µg/ml and 1.953µg/ml respectively. The details of these results are shown in Table 2.

## DISCUSSION

Oral ofloxacin and levofloxacin are widely used in Nigeria with several new brands introduced into the Nigerian market in recent times. Variety of drugs in circulation often put clinicians and pharmacists into difficult situation of choice, and the possibility of interchangeability among brands. The question remains whether these brands can be substituted especially considering that Nigerian drug market are filled with sub-standard products. This formed the major intent of our study which tried to establish the bioequivalence of ofloxacin and levofloxacin marketed in Nigeria. Percentage cumulative quantity of drug recovered in urine after 36 hours for the five brands of ofloxacin and the three brands of levofloxacin were not significantly different. However, percentage cumulative quantity of drugs obtained in our result is less than what has been reported in literature. It has been reported that the recovery of a median range cumulative quantity of ofloxacin 84.3% (46.5% - 92.5%) in 144 hours [13]. The low quantity recovered in this work may be due to the shorter time of observation.

The five brands of ofloxacin and the three brand of levofloxacin compared in our study had the same minimum inhibitory concentration (MIC). This is an indication that the brands were all very potent. Our results showed a higher MIC compared to another study which reported MIC range of 0.25µg/ml to 32µg/ml for ofloxacin and 0.2µg/ml or less of levofloxacin all against *Staphylococcus aureus* [13, 14].

The result obtained in our study is impressive compared to some other bioavailability studies conducted in Nigeria. A study conducted in Nigeria reported that three of the five capsule samples from dispensing points were found to be of

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lower quality than the officially prescribed standards of pharmaceutical quality [15]. However currently published bioavailability studies conducted in Nigeria show some marked improvement in terms of bioequivalence of the same brands of drugs available in the drug market. For example, a recently published study showed that five of the seven brands of metronidazole were physically and chemically equivalent and could be interchanged irrespective of the brands, while two could not [16]. This might be as a result of the intense campaign that has been carried out by National Agency for Food, Drug Administration and Control (NAFDAC) in the country to sanitize the drug market. However, there is need for constant monitoring of new brands of drugs introduced into the drug market to ascertain bioequivalence and conformity with set standards.

## CONCLUSION

The various brands of ofloxacin ($X_1 - X_5$) and levofloxacin ($Y_1 - Y_3$) in Nigeria exhibited same bioavailability data *in vivo* and can be said to be bioequivalents. This indicates that a low price product does not necessarily imply poor quality, and the brands can be prescribed interchangeably. This is an important pharmacoeconomics and essential drug principles. Also the mean cumulative quantity excreted in urine for the various brands and the maximum excretion rates do not show any statistical significant difference. The various brands of both ofloxacin and levofloxacin exhibited maximum excretion rate at 6 hours post administration. Therefore, the various ofloxacin and levofloxacin brands can be said to be bioequivalents.

**CONFLICT OF INTERESTS** The authors declare no conflict of interests.

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