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Invitro comparison of dissolution profiles and antimicrobial activity of three brands of amoxicillin from Mangalore

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ABSTRACT

To know whether the generic formulations of Amoxicillin Trihydrate is as effective as branded formulation. A branded, generic branded and generic version of Amoxicillin trihydrate dispersible tablets were procured and coded as A, B and C. They were compared for the disintegration time, dissolution time and in-vitro antimicrobial activity. The disintegration time was measured using the disintegration apparatus. The dissolution rate (0.1 N HCl at 37^{0}C) was measured using USP2 paddle apparatus. The antimicrobial activity was assessed on E.coli grown on Mueller Hinton agar. The disintegration times were (A) 21 seconds, (B) 51 seconds and (C) 80 seconds. The dissolution rates at 15 minutes were 98.1%, 95.7% and 83.9%. The zone of inhibition was found to be (A) 2.9 cms, (B) 2.6 cms and (C) 2.2 cms. The results show that the 3 products are similar and interchangeable.

INTRODUCTION

Originator brand medicines generally cost substantially more than their generic equivalents. Patients purchasing medicines in the private sector pay, on an average, 2.6 times more for originator brand than for their lowest-priced generic equivalent. The branded medicines are usually expensive as they are strongly promoted through doctors and chemists and such promotional costs add to their retail prices[1].Patent drugs are usually given patent protection for 20 years from the date of submission of the patent. This provides protection for the innovator of such drugs to recover the initial costs incurred by him, viz., research, development and marketing expenses, which have gone into developing the new drug. On expiration of the originator product's patent term protection, other manufacturing companies may file submissions to regulatory authorities for approval to market generic versions of the originator medicine. Generic drugs may be marketed under the non-proprietary name or as a branded generic. Branded generic drugs have names derived from a combination of the manufacturer's name and the non-proprietary name. This enables the manufacturer to market the product in a way similar to the proprietary product[2]. As a consequence of increasing restrictions on the economic resources allocated to public health programmes, many governments strongly support the production and clinical use of generic medicinal products in place of reference brand-name drugs[3].The use of generics is often promoted in the public and private sectors to reduce medicine costs, and increase product availability and consumer access[4]. When generic medicines are of assured quality and are offered at lower prices than the corresponding originator brand product, there is a potential for patients and health systems to achieve equivalent health outcomes at a lower cost. Savings made by using generic medicines allow more patients to be treated with the same amount of money and mobilizes fund to finance other treatment modalities[5]. There are only rare circumstances where substituting a generic drug for a brand name product (or vice

versa) may not be appropriate for a particular patient. For some patients, generic substitution may be inappropriate due to reactions to inactive ingredients or problems with the pill shape, colour or related characteristics[6].

Bioavailability of a drug may be regarded as the quantity of the administered doses, which arrives in a suitable form and concentration at sites within the body where it will exert its biological effect. The FDA's formal definition of bioequivalence is the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study[7].Small difference in the manufacturing process could consistently alter the disintegration, dissolution and consequently the bioavailability of the active ingredients in a product [8].

Disintegration time and dissolution time are two important determinants influencing bioavailability. The development of in-vitro model systems to predict the pharmacokinetics of drugs has become increasingly important. In some instances, guidelines support the use of only in-vitro dissolution studies to test the bioequivalence of generic drugs formulated as oral fast-release tablets without any need for clinical pharmacokinetic or pharmacodynamic investigations[9] The clinical effectiveness exerted by tablet formulation depends on at least two factors such as; the drug must be present in the labeled amount and its availability to the body.[10]Dissolution test is one of the in vitro tests usually employed to assess the quality of oral pharmaceutical solid dosage forms such as tablets and capsules. In vitro dissolution tests are used to guide formulation developments, identify critical manufacturing variables, monitor formulation quality from batch to batch, predict the in vivo performances and also serve as a surrogate for bioavailability and bioequivalence [11].

This study was done to ascertain whether there is any significant difference in the determinants of bioavailability and antibacterial activity of generic, branded generic and branded products of Amoxicillin Trihydrate Dispersible tablets.

MATERIALS AND METHODS

Materials:

Three brands of Amoxicillin Trihydrate Dispersible tablets (250mg) were purchased from KS Hegde Charitable Hospital pharmacy, Mangalore. To perform dissolution test, dissolution tester (TDT-O8L) manufactured by Electrolab, India was used and a disintegration time testing apparatus of Basket-rack assembly manufactured by M.C. Dalal and Co., Chennai was used to perform disintegration time. A spectrophotometer (UV 1700) manufactured by Shimadzu Corporation was used. Mueller Hinton agar and plates along with McConkey plates were used for the biological testing.

The amoxicillin tablets used for comparison were coded as:
Product A; Branded, *Product B;* Branded generic, *Product C;* Generic

Methods:

DISINTEGRATION TIME[12]

The disintegration time testing apparatus consisted of a basket-rack assembly, a 1000-mL low-form beaker, a thermostatic arrangement. The temperature was set to 37^0C.1 tablet was kept in each tube of the disintegration apparatus and the disintegration time was noted. The end point of disintegration of tablet was determined by the absence of a definable tablet segment

DISSOLUTION STUDY[13]

Dissolution studies of all tablets were performed using dissolution tester (TDT-08L, ELECTROLAB, INDIA). The dissolution test apparatus consisted of 8 cylindrical vessels with a normal capacity of 1000 ml, a variable speed motor, a paddle, a withdrawal port and a water bath.0.1 N HCl was prepared by adding 8.3ml of 35%HCl into 1L of distilled water. Tablets were added to the 900 ml 0.1 N HCl at 37^0 C , which was stirred with a rotating paddle at 50 rpm. At time intervals of 5 minutes, 5 ml of samples were withdrawn and equal volume of fresh medium prewarmed at same temperature was replaced in to the dissolution medium after each sampling to maintain its constant volume throughout the test. Assay carried out using U.V. spectrophotometer (UV 1700 shimadzu, Japan) at 272 nm. After standardization, the absorbance values of the dissolution test samples collected at different time intervals (5, 10 and 15minutes) were obtained.

Once the absorbance values of branded, branded generic and generic products were obtained, the cumulative % of drug release was calculated using a standard calibration curve of Amoxicillin trihydrate dispersible tablets at 272nm.The descriptive analysis of the cumulative % of drug release of 15 tablets each of branded, branded generic and generic tablets was done using SPSS softwareversion-17 (SPSS Inc., Chicago, IL, USA).

ANTI BACTERIAL ACTIVITY TESTING[14]
15 tablets each of branded, branded generic and generic products of Amoxicillin trihydrate dispersible tablets were tested for their anti bacterial activity.

ATCC E Coli was the strain used for determining the anti microbial activity. It was subcultured in a McConkey plate. 3-5 well isolated colonies of the same morphological type were selected from the agar plate culture. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4-5ml of peptone water. This tube containing peptone water was incubated at 37^0C until it achieved turbidity. After 1 hour, the tube was taken out from the incubator. A sterile cotton swab was dipped into the Inoculum suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The dried surface of Mueller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times rotating approximately 60^0 anticlockwise each time to ensure an even distribution of inoculums. The rim of the agar was finally swabbed.

Innovation and modification of disc diffusion method
In all, three plates were used. After swabbing, 4 wells were punched in each plate. 10µl of the antibiotic solution from each sample was taken from the test tube using a micro pipette and was transferred into three wells. 10µl of 0.1N HCl was transferred into the fourth well taken as control. These plates were incubated for 24 hours at 37^0C. The next day these plates were taken out and the diameter of inhibition was noted.

RESULTS

*Table 1*and *Figure 1*shows the disintegration time, *Table 3*shows dissolution time and *Figure 2* shows the antimicrobial activity among the branded, branded generic and generic drugs. The median disintegration time for branded, branded generic and generic products were 21 seconds, 51 seconds and 80 seconds. Mann Whitney test was applied to find out the difference between the groups which is shown in *Table 2*. The branded product disintegrated faster than the branded generic and generic product. There was significant difference in disintegration time between the groups and p value was found to be very highly significant (<0.001).

At 15 minutes, the mean cumulative percentage of drug release for generic, branded generic and branded product were found to be 98.06%, 95.72% and 83.87% respectively. Branded product showed higher cumulative percentage of drug release compared to branded generic and generic product. There was significant difference in mean cumulative percentage drug release between the products and p value was found to be very highly significant (<0.001).

Evaluation of the anti bacterial activity involved the innovation of the method of anti bacterial activity testing on the agar plates by creating 4 wells with the dropper to replace the use of antibiotic impregnated discs as in case of the standard disc diffusion method. This innovation yielded similar reliable results as in case of standard disc diffusion method. Test for antibacterial activity was conducted using the samples withdrawn at 5, 10 and 15 minutes from the dissolution apparatus.

The zone of inhibition (in cm) was calculated. Branded product showed better zone of inhibition compared to branded generic and generic product. P value was found to be very highly significant (<0.001) between the groups.

For the branded product, the percentage of drug released at 5 minutes was 12.96% which produced a zone of inhibition of 2.273 cm, at 10minutes was 50.71% which produced a zone of inhibition of 2.553cm and at 15 minutes was 98.06% which produced a zone of inhibition of 2.906cm.

For the branded generic product, the percentage of drug released at 5 minutes was 11.66% which produced a zone of inhibition of 1.64cm, at 10minutes was 45.81% which produced a zone of inhibition of 2.12 cm and at 15minutes was 95.72% which produced a zone of inhibition of 2.55 cm.

For the generic product, the percentage of drug released at 5 minutes was 9.49% which produced a zone of inhibition of 1.42cm, at 10 minutes was 40.52% which produced a zone of inhibition of 1.9cm and at 15 minutes was 83.87% which produced a zone of inhibition of 2.22 cm.

Table 1. Median and Inter quartile range for disintegration time

GROUP	MEDIAN(SECONDS)	INTER QUARTILE RANGE(IQR)
BRANDED	**21**	**19 – 25**
BRANDED GENERIC	**51**	**46 – 55**
GENERIC	**80**	**74 – 90**

MANN WHITNEY TEST

Mann Whitney test was applied to find out the difference between the groups.

Table 2 Mann Whitney test was applied to find out the difference between the groups

Between groups	P value(significance)
BRANDED AND BRANDED GENERIC	<0.001*
BRANDED GENERIC AND GENERIC	<0.001*
BRANDED AND GENERIC	<0.001*

**highly significant*

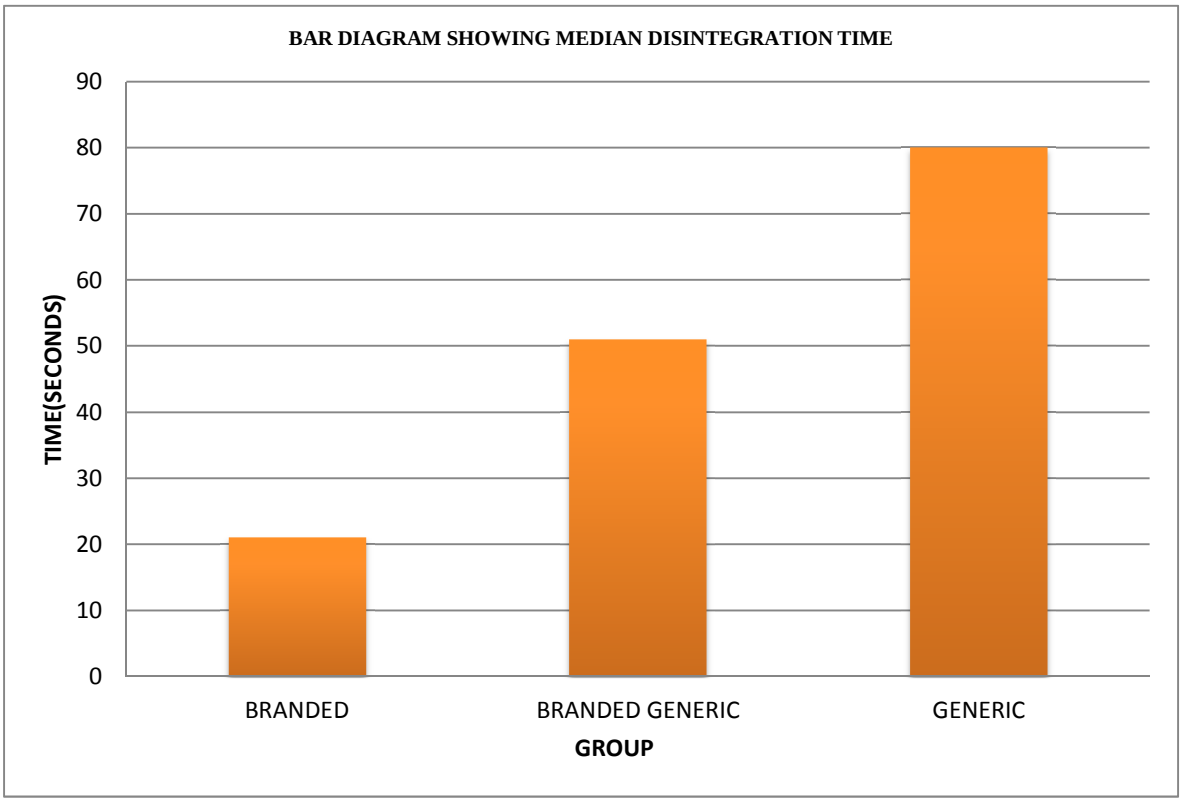

Figure 1 Disintegration time

Table 3 –dissolution study

SL.NO.	TIME(MINUTES)	MEAN CUMULATIVE % OF DRUG RELEASED (MEAN±STANDARD DEVIATION)
PRODUCT A	**5**	**12.96±0.97**
	10	**50.71±0.71**
	15	**98.06±0.96**
PRODUCT B	**5**	**11.66±0.47**
	10	**45.81±1.00**
	15	**95.72±0.86**
PRODUCT C	**5**	**9.49±0.58**
	10	**40.52±0.57**
	15	**83.87±0.89**

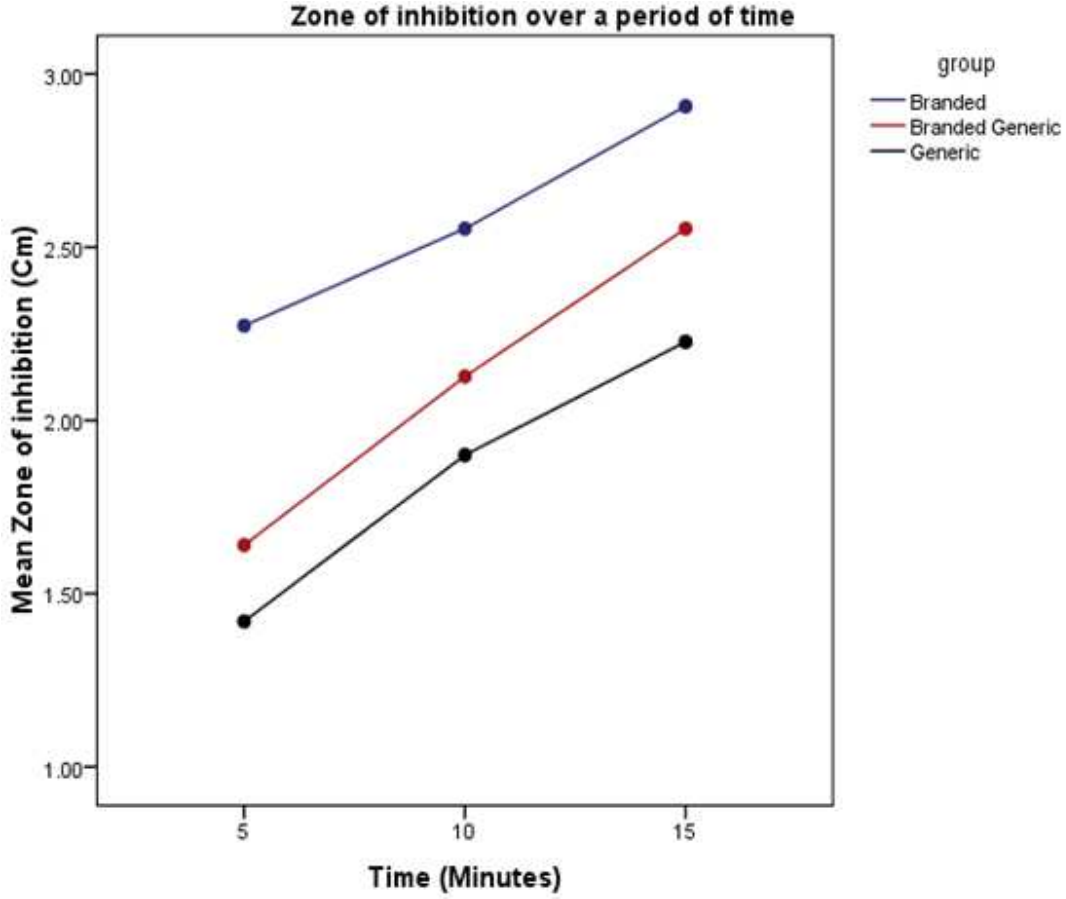

Fig 2 –Antimicrobial study

DISCUSSION

Amoxicillinis semi synthetic, ß -lactam antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms.

It is commonly used in respiratory tract infections, genitourinary tract infections, skin and skin structure infections, tonsillitis, otitis, H pylori, Chlamydial infections in pregnancy, gonorrhea etc[15]

Generic substitution has become a common practice since the late 1970s in the United States. At that time, many of these generics caused bioavailability problems, which fueled suspicions about their efficacy and safety and the Food and Drug Administration (FDA) standards for bioequivalence[16]The 1984 Hatch-Waxman Act first authorized the FDA to approve generic drugs demonstrated to be bioequivalent.[17] The FDA still states on its website that, ‘All

generic manufacturing, packaging, and testing sites must pass the same quality standards as those of brand name drugs and the generic products must meet the same exacting specifications as any brand name product.[18]

For generic antibiotics, differences in pharmaceutical properties might result in changes of their pharmacokinetic profiles with consequent alteration of pharmacokinetic/pharmacodynamic relationships leading ultimately to variations in their clinical efficacy with respect to the brand-name counterparts In effect, poor dissolution of active ingredient can cause a low bioavailability which may lead to therapeutic ineffectiveness firstly and secondly a spread of resistance. Defects in the formulation or manufacturing process may be responsible for the development of generic drugs of poor quality[19]. Spread of spurious/counterfeit/substandard drugs is a modern day menace which has been recognized internationally, especially so in developing countries. The consequences of the use of such medicines may vary from therapeutic failure to the occurrence of serious adverse events and even death[20]Also, improper storage of the pharmaceutical products is one of the fundamental concerns in patient care. The loss of potency during storage may influence the efficacy and safety of pharmaceuticals[21]. There is a possibility that handling and transport can also affect the quality of the product Amoxicillin belongs to BCS class 1 drug[22]. That means it has got rapid solubility and good permeability. Such products are exempted from bioavailability studies, where disintegration and dissolution profiles are considered as surrogate markers for bioavailability and bioequivalence studies.[23]Hence we undertook the present study. In this study generic and branded generic had acceptable dissolution profile whereas the branded generic was a fraction less than the acceptable standard. The in vitro antimicrobial study showed that the efficacy of generic was more than branded and that of branded more than branded generic. This result was comparable to their dissolution profiles.

Hitherto the FDA was laying the guidelines of good manufacturing practice and inspecting the manufacturers' facility for approval to get a trade license and did periodic inspections to ensure quality control .The onus was on the manufacturer. But there has been a hue and cry in the USA because of the insurance policy of reimbursement of cheaper generic drugs only claiming that the generics were inferior to the originator brand. Therefore the FDA has introduced new testing programs by affiliating various academic institutions for generic drug testing[24].We strongly opine that similar strategy can be implemented in India, especially because of the additional problem of counterfeit and spurious products.This would improve the quality of generics used as a substitution for branded drugs.

CONCLUSION

We conclude that there is a need to cross check the quality of life saving pharmaceutical products. Such a measure can be implemented systematically as a routine with the help of academia. This will enable to improve the health care system.

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