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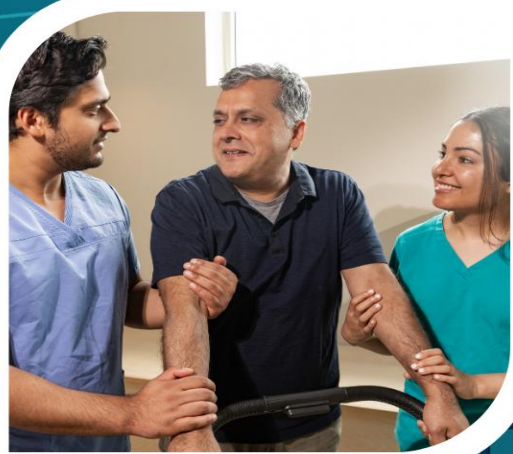

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Assessment of the In Vitro and In Vivo Quality of Glibenclamide Tablets Sold in Addis Ababa, Ethiopia

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1. Introduction

A effective course of treatment requires high-quality medications. Nowadays, drug quality is gaining more and more attention on a global scale. The public has become more aware over the last ten years of the presence of counterfeit and inferior medications, which are more frequently reported in developing nations with lax or nonexistent drug laws [1]. The use of subpar products can result in unforeseen adverse effects, antimicrobial resistance, increased morbidity and mortality, treatment failure, and a decline in public trust in healthcare [2].

Numerous issues have arisen when generic medicine goods from various sources were introduced into the health care delivery systems of many developing nations. The most significant of these issues is the extensive circulation of counterfeit and inferior drug products [3]. Bioequivalence studies become crucial in order to help replace branded innovator products with generics for cost-effectiveness while also achieving therapeutic efficacy [4].

To guarantee that there are no notable variations in the rate and degree to which the active components become available at the site of therapeutic action when taken under comparable circumstances and via comparable routes, bioequivalence studies are crucial for generic drugs [5]. The same quality, effectiveness, and safety requirements that apply to innovator goods also apply to generic medicine products [6]. Both in vitro and in vivo research may be used in bioavailability or bioequivalency investigations [7].

Type II diabetic mellitus (DM) is frequently treated with glibenclamide, also referred to as glyburide, an oral hypoglycemic medication of the sulphonylurea class [8]. By encouraging the release of insulin from the pancreatic beta cells, it reduces blood glucose levels [9]. Following the expiration of the innovator brand's patent, glibenclamide pills are now accessible in a number of generic forms throughout the world. There are published studies that compare the in vitro quality assessment of generic glibenclamide pills from various nations. El-Sabawi et al. [10] investigated five glibenclamide tablet generics that were sold in

TABLE 1: Detailed description of products of glibenclamide tablets included in the study.

Brand	Manufacturer	Batch Number	Mfg. date	Exp. date
Betanase	Cadila Health Care Limited (India)	GM3429	12/2012	11/2016
Daonil	Sanofi Aventis (France)	3AP9A	03/2013	03/2015
Glamide	Cadila Pharmaceuticals PLC (Ethiopia)	D12010BX75	09/2012	08/2016
Glitisol	Remedica Ltd. (Cyprus)	52994	10/2012	10/2017
Melix	Lagap SA (Switzerland)	B050	05/2011	04/2016

According to the Jordan market, they showed dissolution profiles that differed greatly from the original Daonil's and from each other. Furthermore, Elhamili et al. [10] assessed three brands of glibenclamide pills that were sold in Libya and found that every product met British Pharmacopeia (BP) requirements. The growing usage of glibenclamide tablets in clinical practice makes it necessary to keep an eye on and determine the quality of the many brands that are offered on the pharmaceutical market. In Ethiopia, oral glibenclamide is extensively used, and in recent years, a number of new brands have entered the Ethiopian market. Because of the wide range of medications on the market, doctors and pharmacists frequently face a challenging decision regarding brand interchangeability [12, 13]. Using inferior products can result in poor blood glucose control, which can lead to potentially fatal complications. Although glibenclamide is widely used to treat non-insulin dependent diabetic mellitus (NIDDM) in Ethiopia, no data have been found about the bioavailability and bioequivalence of the different brands in the nation. Therefore, the current study was conducted to evaluate the physicochemical bioequivalency and quality of glibenclamide pills that are sold in Addis Ababa, Ethiopia.

2. Supplies and Procedures

2.1. Supplies. Several kinds of 5 mg glibenclamide tablets were purchased from different pharmacies in Addis Ababa, Ethiopia's capital. At the time of the investigation, every brand was within its shelf life. Table 1 presents these goods' comprehensive descriptions. The United States Pharmacopoeia (USP) reference standard for glibenclamide was acquired from the Ethiopian Food, Medicines and Health Care Administration and Control Authority (EFMHACA). Every chemical that was used was analytical grade.

2.2. Solubles and Reagents. 85% phosphoric acid (Riedel-de Haen, Germany), potassium phosphate monobasic (Fisher Scientific, USA), sodium hydroxide (BDH limited, Poole, England), distilled water, monobasic ammonium phosphate (FARMITALIA CAROERBA, Italy), and HPLC grade acetonitrile (BDH Laboratory Supplies, England) were the chemicals and reagents used in the experiments.

2.3. Tools and apparatus. For the experiments, the following tools were utilized: balance of analysis (Mettler Toledo, Switzerland), a hardness tester (Schleuniger, 2E/205, Switzer-land), a friability tester (ERWEKA, TAR 20, Germany), a disintegration apparatus (CALEVA, G.B. Caleva Ltd., UK), a dissolution apparatus (ERWEKA, DT600, Germany), a UV-visible spectrophotometer (Single beam spectrophotometer, CM2203, Belarus), filter paper (diameter 110, lot ER0692-1, Schleicher and Schell, Germany), a vacuum filter (Scientific Laboratories Supplies, Germany), a glucometer (Prodigy Diabetes Care LLC, USA), a PH meter (Mettler Toledo, Switzer-land), and an HPLC-UV (Shimadzu Corporation, C18 stainless steel column (15 cm x10 mm), a UV-VIS detector, Japan).

2.4. Animals used in experiments. The Department of Biology at Addis Ababa University provided Swiss albino mice of either sex, measuring 21–30 g and aged 5–6 weeks. Every animal was kept in a room with air conditioning. Standard pellets and unlimited water were provided to them. One week before the studies began, the animals were conditioned.

2.5. Approaches. Pharmacopeial methodologies outlined in USP/NF XXIV, 2000 [14], USP/NF 25, 2007 [15], and BP, 2009 [16] were used in drug quality evaluation tests.
2.5.1. Test of Hardness. Using a hardness tester, six tablets were chosen at random to determine each tablet's hardness. The crushing strength that breaks a tablet was measured after each tablet was positioned between two anvils and force was applied to the anvils. An average of six pills' crushing strength was noted.
2.5.2. Test of Friability. An analytical balance was used to weigh ten tablets of each brand. The tablets were inserted into the friability tester's drum and rotated for four minutes (100 times) at a rate of 25 revolutions per minute (rpm). Tablets were then weighed and dedusted. After comparing the weights to their starting weights, the weight difference was used to compute the percentage of friability.
2.5.3. Time of Disintegration. The USP/NF (2007) specification is followed when conducting the disintegration time test. At 37 0.5°C, six pills were put in a disintegration tester with distilled water. When every particle had gone through the wire mesh, the tablets were deemed fully disintegrated. The disintegration time was measured in minutes.

TABLE 2: Results of hardness, friability, disintegration tests, and chemical assay of different brands of glibenclamide tablets included in the study.

Drug Product	Brand	Hard ness (N) ± SD	Friability (%)	Disintegration time ±SD (Min)	Assay (%W/W ±SD)
Glibenclamide 5 mg	Betanase	65.83±6.79	0.224	2.83±0.25	101.05±0.16
	Daonil	81.66±4.5	0.106	1.43±0.14	95.53±0.54
	Glamide	50.3±2.65	0.289	1.36±0.09	100.68±1.83
	Glitisol	85.5±3.39	0.105	6.44±0.44	104.33±0.04
	Melix	101.66±3.38	0.126	2.29±0.19	97.32±0.65

Chemical Assay 1.1.1. Glibenclamide was assayed using the BP (2009) technique employing high pressure liquid chromatography (HPLC) with a UV/VIS detector and a C18 stainless steel column (15 cm x 10 mm). Acetonitrile and a 1.36% w/v solution of potassium dihydrogen orthophosphate (previously adjusted to pH 3.0 with 85% orthophosphoric acid) at a ratio of 47:53, with a flow rate of 1.5 ml/min, made up the mobile phase composition. The wavelength was fixed at 300 nm, and the sample injection volume was 20 l.

Preparing a sample. Weighed and powdered were twenty pills. With the use of an ultrasonic bath, a quantity of the powdered tablets containing 5 mg of glibenclamide was combined with 2 ml of water and 20 ml of methanol, and the combination was then filtered through a vacuum filter.

standard preparation. With the help of an ultrasonic bath, 50 mg of the Glibenclamide working standard was dissolved in 10 ml of methanol for 20 minutes. Enough methanol was then added to create 50 ml of stock solution, and the resultant solution was then diluted to 200 ml with methanol. The assay preparation and the standard preparation were separately injected into the HPLC system after serial dilutions of the stock solution were made to achieve calibration concentrations of 181.8181 g/ml, 204.5454 g/ml, and 227.2727 l). The chromatograms were then recorded, and the peak areas were obtained to be used for amount calculation.

1.1.2. Test of Dissolution. The USP/NF XXIV, 2000 [14] specification was followed for dissolving six tablets of each brand using a dissolution device type II (paddle apparatus) at a rate of 50 rpm and 37 0.5°C. At 10, 20, 30, 45, and 60 minutes, 10 ml of the sample was removed, and the same volume of brand-new dissolving medium was added. Following the proper dilution of the filtered samples, absorbance measurements were made using a UV/Visible Spectrophotometer set to 226 nm. Each sample's concentration was ascertained using the calibration curve. Each time, the percentage of medication release was computed.
standard preparation. A 100 μg/ml stock solution was pre-

For the working standard solution, 0.01 mg/ml was obtained by diluting the solution with phosphate buffer to 100 ml. Ultimately, 16 ml, 20, 24, 28, 32, and 36 ml of the resultant solution were pipetted out sequentially into a 50 ml volumetric flask. These volumes were adjusted to obtain concentrations of 0.0032, 0.004, 0.0048, 0.0056, 0.0064, and 0.0072 mg/ml, respectively. The UV-Visible Spectrophotometer was used to detect the absorbance at 226 nm.

1.1.3. Check for the hypoglycemic effect. There have been several investigations on the antidiabetic effects of plant extracts in normoglycemic rats [17, 18]. In the current study, the hypoglycemic effect of glibenclamide products on normoglycemic mice was investigated using the methodology outlined by Saidu et al. [17].
Swiss albino mice of both sexes were kept in stainless steel cages at random and given regular pellets and unlimited access to drinking water. A week before the tests began, the animals were acclimated. The animals were then split up into six groups, each consisting of five mice. Different brands of glibenclamide pills (5 mg/kg p.o.) were administered to groups 1–5. As a control, animals in group 6 were given 90% Dimethyl Sulfoxide (DMSO). Prior to the trial, all groups were required to fast for sixteen hours. For oral treatment, glibenclamide pills in powder form were dissolved in DMSO. Each group's tail tips were cut to obtain blood samples at 0, 1, 2, 3, and 4 hours. A glucometer was then used to assess the mice's blood glucose levels [19, 20].

1.2. Analysis of Data. ORI-GIN® graphing and scientific analysis tools, Microsoft Excel 2007 and Windows SPSS Version 20 were used to process the data. One-way analysis of variance (ANOVA) was used to determine statistical significance and correlation. A 95% confidence interval (P 0.05) was used to assess all of the data.

2. Findings and Conversations
2.1. Friability and Hardness Test. Table 2 displays the average hardness value of glibenclamide tablets. According to the findings, the brands' mean hardness values fell between 101.6 and 6.38 N, with Glamide having the lowest hardness.
Glibenclamide working standard (50 mg) was dissolved in 500 ml of phosphate buffer (pH 7.4), and 10 ml of the resultant requirement for tablet hardness that is acceptable [21]. Therefore, the glibenclamide tablets of all manufacturers had adequate

TABLE 3: Time dependent drug release of different products of glibenclamide tablets.

sampling time (min)	Percent of drug release (W/W) ±RSD				
	Betanase	Glitisol	Glamide	Melix	Daonil
10	52.77± 0.18	60.25±0.031	38.95±0.09	44.94±0.04	51.89±0.028
20	60.06±0.003	62.67±0.032	46.93±0.2	54.85±0.006	53.64±0.012
30	62.18 ±0.02	64.5±0.018	50.99±0.1	67.5 ±0.067	56.79± 0.06
45	64.65 ±0.28	67.358±0.01	54.57±0.07	70.13± 0.04	60.698±0.02
60	66.45± 0.01	70.34±0.019	58.88±0.08	73.16±0.035	61.57 ±0.072

hardness. Likewise, Table 2 displays the tablets' weight loss percentage following the friability test. The findings demonstrated that the friability values of all glibenclamide tablet brands ranged from 0.105 to 0.106%. Glamide (0.289) and Glitisol (0.105) have the lowest and maximum friability, respectively. Tablet friability should be less than 1%, according USP (2007). As a result, every brand met the friability requirement.

One crucial factor that aids in determining a tablet's resistance to breaking under handling, storage, and transportation conditions is its hardness or crushing strength [22]. Therefore, it's critical that tablets have the ideal level of hardness [23]. The purpose of the friability test, which is closely linked to tablet hardness, is to assess the tablet's resistance to abrasion during coating, packaging, handling, transportation, and other manufacturing processes [24]. For tablets to be accepted by consumers, they typically need to be sufficiently hard and reasonably friable [25].

The disintegration test (1.1). Table 2 displays the average disintegration time for the various glibenclamide tablet brands. The findings demonstrated that every brand passed the USP (2007) disintegration test, which calls for 30 minutes for tablets that are uncoated and film-coated. With the exception of Glitisol, all glibenclamide tablet products had typical disintegration times of less than five minutes. The kind and quantity of disintegrant employed in the formulation may be the cause of the quick disintegration time displayed by all the brands. Glitisol's low friability and high hardness ratings are correlated with its longest disintegration time (6.44 0.44).

Before the medication may dissolve and be absorbed from its dose form, the tablet must disintegrate. A medication that is included in a tablet will be delivered quickly as Table 2 shows the mean percentage label claim results for the various brands of glibenclamide tablets. The products were analyzed using the procedure described in BP (BP, 2009). Glibenclamide tablets should contain at least 95.0% and no more than 105.0% of the specified amount, according to BP (2009). All goods met the pharmacopeial requirements for the percentage content of active ingredient, as shown in Table 2. Glitisol had the greatest percentage content (104.33%), while Daonil had the lowest drug content (95.53%).

The assay of tablets guarantees the amount of active ingredient, which is indicative of the product's stability and efficacy. Tablets contain a specific amount of active ingredient with a permitted variable limit. Daonil's drug content differs significantly from that of all other brands of glibenclamide, according to a statistical comparison with a 95% confidence interval (P 0.05).

3.4. Test of Dissolution. Daonil was the originator of the five glibenclamide tablet formulations that were examined. The dissolution curves of the test and innovative products were merged based on mean percentages of medication released in order to compare the dissolution profiles; these are shown in Table 3 and Figure 2.

Figure 2 shows that not a significant fraction of the medicine was released in the first half hour by any of the products, including the innovative product Daonil. In actuality, Glamide and Daonil released roughly 50% of the medicine in 30 minutes, while Betanase, Glitisol, and Melix released over 60% of the drug in

the same time.
To compare the dissolution characteristics of the various glibenclamide tablet products, the model independent parameters, similarity factor () and difference factor (f1), were computed from the dissolution profiles [27].

The tablet breaks up. The disintegration rate has an impact on the

$\sum n$

-

T

disintegration and, in turn, the effectiveness of the

$f1$ =(

t=1 n t

t) × 100, (1)

medication [12]. Disintegration test results are known to be impacted by many formulation parameters. The kind and quantity of excipients utilized in the manufacturing of tablets, as well as the

f = 50 ×

$\sum t$=1 Rt

[100]

It is well recognized that the turving process influences both the disintegration

2 log10 [n

2] and (2)

and conditions for dissolution [26].

Chemical Assay (1.2). The concentration of the active medicinal ingredient in a sample is ascertained using the test. HPLC was used for the test. The averages of the peak regions were computed after injecting samples. Figure 1 displays representative peaks.

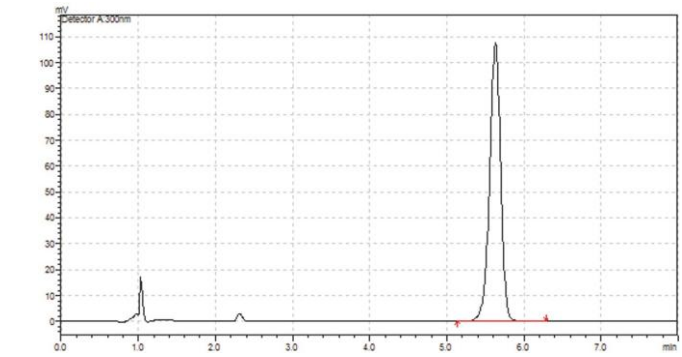
$\sqrt{1+}$ $\sum t$=1 $(Rt - Tt)$ $/n$

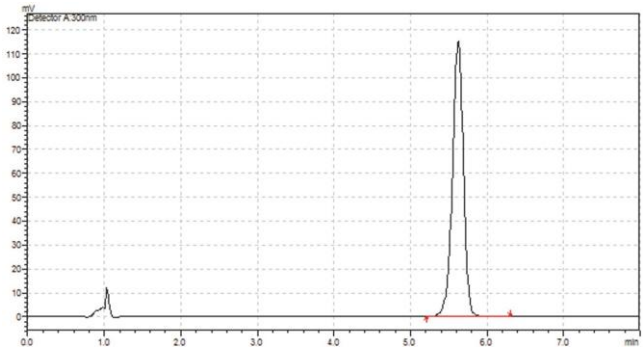
where n is the number of time points and Rt and Tt are the cumulative percentage of dissolved medication for the reference and test formulation at time t, respectively. These guidelines generally state that

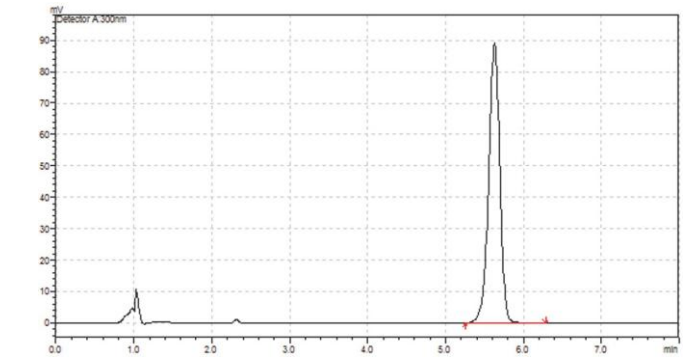
resemblance or equivalency between the two is ensured by f1 values up to 15 (0–15) and f2 values larger than 50 (50–100).

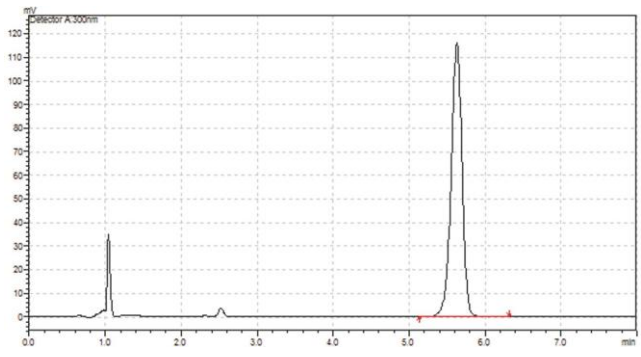
TABLE 4: Similarity and difference factors of glibenclamide products.

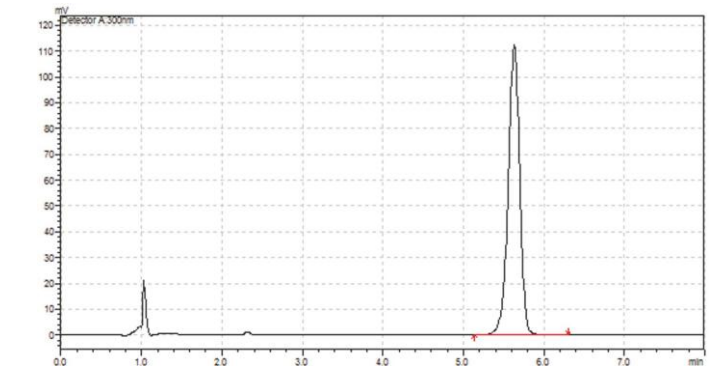
Products	Betanase	Glamide	Glitisol	Melix
f1	7.56	12.04	14.24	14.02
f2	65.912	55.695	54.26	52.61


Chromatogram of Glibenclamide standard


Chromatogram of Daonil tablets


Chromatogram of Glamide tablets


Chromatogram of Betanase tablets


Chromatogram of Melix tablets

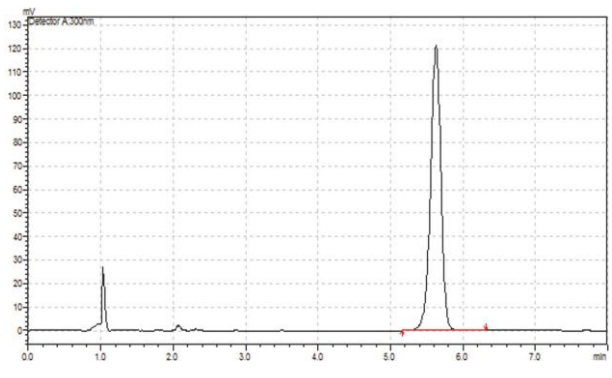

Chromatogram of Glitisol tablets

FIGURE 1: Typical HPLC chromatogram's obtained in the assay.

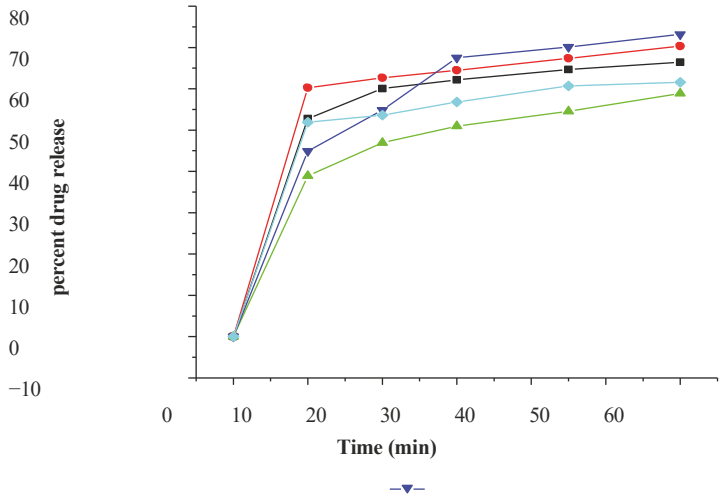
profiles of disintegration [28]. Table 4 shows the f1 and f2 values that were computed between the test products and the reference product (Daonil).
For all glibenclamide products, as seen in Table 4, the f1 values are less than 15 and the f2 values are larger than 50, indicating that the dissolution profiles are similar or equivalent. This demonstrated that the release of glibenclamide from all products was comparable to the reference and validated the comparability between all glibenclamide products when compared to the innovator product (Daonil).
3.4. Research in Vivo

3.4.1. Glibenclamide Tablets' Hypoglycemic Impact on Normo-glycemic Mice. One method of determining a drug's bioavailability is to quantify its pharmacologic effect. This approach is predicated on the idea that a specific drug concentration at the site of action corresponds to a given response intensity [7].

In mice given glibenclamide orally, Tmax is two hours [29]. Table 5 indicates that Glamide (45.99%) demonstrated

TABLE 5: Percent reduction in blood glucose level of normoglycemic mice for glibenclamide products.

Brand	Percent reduction in blood glucose level (mg/dl) ± SD			
	1 h	2 h	3 h	4 h
Betanase	31.55±9.52	36.27±14.69	30.15±6.07	30.4±16.56
Daonil	17.14±10.98	40.58±11.65	50.71±9.54	33.81±18
Glamide	43.16±13.87	45.99±20.65	46.7±15.43	50.38±7.4
Glitisol	40.57±10.9	44.62±9.48	47.53±5.81	52.22±3.28
Melix	33.93±23.33	38.77±11.16	37.92±7	37.29±8.83



of manufacturing these tablets, effective control activities of EFMHACA, and the proper storage of these drugs by wholesalers, retailers, and pharmacies.

2. Conclusion

This study assessed quality as well as physicochemical bioe- quivalence of five brands of glibenclamide tablets marketed in Addis Ababa using *in vitro* and *in vivo* methods. The study confirmed that brands of glibenclamide tablets complied with the official specification for hardness, friability, assay, and disintegration. The f1 values were less than 15 and f2 values were greater than 50 for all products of glibenclamide. This suggests that release of the drug from all products of glibenclamide is similar to the innovator product. In addition,

Betanase Glitisol Glamide

Melix Daonil*In vivo* studies of the products of glibenclamide tablets indicated that there is no significant difference in percent reduction of blood glucose level between Daonil and the

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FIGURE 2: Dissolution profiles of different products of glibenclamide tablets.

highest percent reduction in blood glucose level at T_{max} while the least was observed for Betanase with 36.27 % at T_{max} (2 h).

Statistical analysis was conducted using Dunnett's t-test and it was found that, at 95% confidence interval, there was no significant difference in the percent reduction of blood glucose level between Daonil and Glitisol, between Daonil and Melix, and between Daonil and Betanase at l h (p 0.05), while Glamide was significantly different from the innovator product. Glamide showed highest reduction in blood glucose level compared to the innovator product. At 2 h (T_{max} value) and 4 h, there was no significant difference between Daonil and all the other brands of glibenclamide (p 0.05), suggesting that the serum blood glucose profiles generated by the reference tablets were comparable to those produced by the test products. Hence, based on *in vivo* results and *in vitro* dissolution studies, the brands might be substituted with the innovator product in clinical practice.

From both *in vitro* and *in vivo* studies, it was shown that there are minor variations among the generic products of glibenclamide tablets. Despite that, all the studied gliben- clamide tablets distributed in the Ethiopian market are of good quality products. This might be as a result of strict adherence to good manufacturing practice in the process other brands (p 0.05). Hence, based on the *in vivo* results and *in vitro* dissolution studies, any of the glibenclamide products might be substituted with the innovator product in clinical practice.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors in this manuscript did not have any conflicts of interest to declare.

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