TECHNICAL NOTE

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Effect of *Hibiscus Sabdariffa* (Calyxes) Water Extract on the *In Vitro* Availability of Lisinopril

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract
Background: The concurrent use of herbs and drugs for the treatment of various ailments is a common practice amongst patients; a practice that could result in drug-herb interaction.
Objectives: This study is aimed at evaluating the effects of *Hibiscus sabdariffa* on *in vitro* availability of lisinopril.
Method: The availability of lisinopril alone and in presence of *Hibiscus sabdariffa* calyxes water extract was determined using dissolution apparatus (BP, 2013) set at 50 rpm and 37 °C in 900 mL of three different dissolution media [0.1 M HCl (simulated gastric pH), phosphate buffers pH 6.8 (simulated intestinal pH) and phosphate buffers pH 7.4 (simulated blood pH)]. Samples (5 mL) were withdrawn from the media at 5, 10, 15, 30, 45, and 60 min and replaced immediately with the same medium. Each sample was analysed for the lisinopril content released using UV spectrophotometry at 215, 210 and 215 nm in 0.1 M HCl, phosphate buffers pH 6.8 and phosphate buffers pH 7.4 respectively.
Results: Results showed that the media has no effect on the dissolution profile of lisinopril alone, however, it was observed that 89.40 (lisinopril alone) and 92.62 % (lisinopril in the presence of *Hibiscus sabdariffa*) was released in simulated gastric pH. The corresponding contents of lisinopril observed in simulated intestinal pH were 89.40 and 92.51 %, while in simulated blood pH 89.40 and 91.95 % of lisinopril was released. The presence of *Hibiscus sabdariffa* significantly ($p < 0.05$) increased the *in vitro* availability of lisinopril in all the media.
Conclusion: The results of this study suggest that coadministration of lisinopril with *Hibiscus sabdariffa* could enhance its *in vitro*availability consequent to the increased dissolution of lisinopril in simulated gastric, intestinal and blood pH.
Keywords: Lisinopril, Interaction, *Hibiscus sabdariffa*, dissolution.

INTRODUCTION

Lisinopril (Figure 1) is a metallopeptidase antihypertensive that acts by inhibiting angiotensin converting enzyme. It is a white to almost white crystalline powder in appearance, soluble in water, sparingly soluble in methanol, practically insoluble in acetone and in anhydrous ethanol (BP, 2013). Lisinopril is slowly and incompletely absorbed after oral doses. About 25% of a dose is absorbed on average, but the absorption varies considerably between individuals, ranging from about 6 to 60%. It is already an active diacid and does not need to be metabolised *in vivo* to elicit its effects. The time to reach peak plasma concentration is reported to occur after about 7 hours. Lisinopril is not significantly bound to plasma proteins and is excreted unchanged in the urine (Sweetman, 2002).

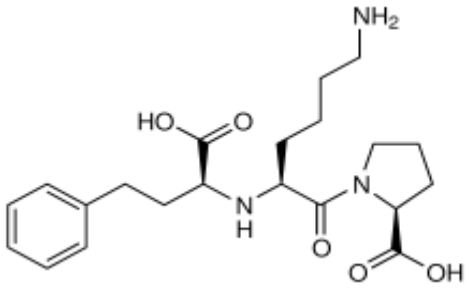

Figure 1: Chemical structure of lisinopril

Drugs have a great potential for interactions with other drugs, foods, herbs and diseases. These interactions may be beneficial or harmful (Amorha *et al*., 2013). Although, the medical literature is awash with case reports of adverse drug interactions, most of which are clinically significant (Sweetman, 2002; Izzo and Ernst, 2009; Kumar *et al.*, 2011; Chen *et al.*, 2012; Hopkins *et al*., 2013). Thus, it is important to anticipate when a potential drug interaction might have clinically significant consequences for the patient so that advice may be given to minimize the risk of harm. This may be achieved by avoiding the combination, making dosage adjustments, spacing dosing times, close monitoring of patient (Lee and Stockley, 2007).

Hibiscus sabdariffa is well known for its edibility and medicinal properties (Ismail *et al.*, 2008; Bako *et al*., 2009; Bolade *et al*., 2009), though the calyx is the most frequently used portion of the plant (Okekere *et al*.,2015).

A preparation of *Hibiscus sabdariffa* calyxes water extract popularly called 'Zobo' is a widely used food drink in northern Nigeria. The extracts of *Hibiscus sabdariffa* are generally considered to have a low degree of toxicity with LD_{50} above 5000 mg/kg leading to widespread use (Allison *et al*., 2013). *Hibiscus sabdariffa L.*, extracts have been reported to show antibacterial, antioxidant, nephro and hepato-protective, renal or diuretic effect, anti-cholesterol, anti-obesity, antidiabetic and antihypertensive effects among others (Ojeda *et al*., 2010; Mossalam *et al.*, 2011; Peng *et al.*, 2011; Inuwa *et al*., 2012; Perez-Torres *et al.*, 2012; Da-Costa-Rocha *et al*., 2014).

These pharmacological reports along with traditional claims of its medicinal efficacy have resulted in the concurrent use of *Hibiscus sabdariffa* with orthodox medicines by many people (Nuria and Guardiola, 2014). *Hibiscus sabdariffa* water extract is commonly taken concurrently with lisinopril especially in people with hypertension probably due to its reported antihypertensive effect (Jacoba*et al*., 2004; Olisa and Oyelola, 2009). This practice may result in potential drug-herb interaction (Chen *et al.*, 2012). Therefore, the present study was aimed at evaluating the effect(s) of *Hibiscus sabdariffa* (calyxes) water extract on the *in vitro* availability of lisinopril.

METHODOLOGY

MATERIALS AND METHODS

Equipment and Reagents

Standard lisinopril powder (Sigma Aldrich, Germany). Lisinopril tablets (Zestril®) 10 mg (Batch No: LT626). Potassium dihydrogen orthophosphate was BDH-Laboratory reagents. Dissolution machine 3 in 1 78×2Btype (Huang Hai Medicament Testing Instrument Company Limited, Shanghai). DENVER INSTRUMENT APX-200 digital weighing balance. A double scanning UV/VIS spectrophotometer (Model SP3000) was used to monitor the drug content.

Preparation of Dissolution Media

Dissolution was carried out in simulated gastric juice (containing 0.1M hydrochloric acid), intestinal pH (phosphate buffer pH 6.8) and blood pH (phosphate buffer pH 7.4). All media were prepared using official methods (BP 2013).

Collection, Identification and Preparation of *Hibiscus sabdariffa* Calyxes

The plant sample of *Hibiscus sabdariffa* was collected during the month of December, 2016. It was identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria by Malam Namadi Sanusi and was assigned a voucher number of 1056. The calyxes were removed, shed dried and size reduced and kept in air tight container for subsequent use.

Preparation of Hibiscus sabdariffa extracts in the media (1 g/L)

Extracts of *Hibiscus sabdariffa* were prepared by weighing a quantity (5 g) of the dried size reduced calyxes and macerating in 1 L of each of the media [0.1 M HCl (simulated gastric pH), phosphate buffers pH 6.8 (simulated intestinal pH) and phosphate buffers pH 7.4 (simulated blood pH)] for 24 hr. Each extract was filtered and a portion of the filtrate (100 mL) was evaporated to dryness using water bath at 60°C. The extract was weighed and the concentration was found to be 0.25 g/100 mL. A quantity of each of the extracts (400 mL) was prepared and diluted to 1 L to obtain solutions containing 1 g/L for each of the media

Identification and Assay of Lisinopril

Lisinopril standard powder and the tablet brand used for this study were both identified and assayed using official methods (BP, 2013).

Analytical Method

The method for determination of lisinopril content in the three media (0.1 M HCl, phosphate buffer pH 6.8 and 7.4) reported by Nasir *et al.* (2017) was adopted for this study. Stock solutions (100 µg/mL) of lisinopril were prepared in each of the three media (0.1 M HCl, phosphate buffer pH 6.8 and 7.4). Calibration curves of lisinopril in the media were prepared by serially diluting each stock solution to obtain solutions in the concentration range 2.5 to 15.0 µg/mL;whose absorbance were measured at 215, 210 and 215 nm corresponding to λ_{max} of lisinopril in 0.1 M HCl, phosphate buffer pH 6.8 and 7.4, respectively (Nasir *et al*., 2017).

Availability of Lisinopril

The *in vitro* availability of lisinopril in simulated gastric, intestinal and blood pH at 37ºC were determined using dissolution apparatus as outlined in BP (2013) with slight modification to the top of the basket in order to prevent air entrapment during dissolution. A tablet of lisinopril (10 mg) was placed in the dissolution basket, allowed into the dissolution medium (900 mL) and operated at 50 rpm (BP, 2013). The dissolution process was monitored over a period of 60 minutes, with aliquots (5 mL) being withdrawn at 5-, 15-, 30-, 45- and 60-minute intervals and the drug content available in the aliquots were determined by taking the absorbances of the withdrawn samples directly without dilution at215, 210 and 215 nm corresponding to λ_{max} of lisinopril in 0.1 M HCl, phosphate buffer pH 6.8 and 7.4 respectively (Nasir *et al.,* 2017). The volume of the dissolution medium was maintained after each withdrawal by an immediate replacement with 5 mL of the dissolution medium maintained at the same temperature in the same bath.

For the *in vitro* availability of lisinopril in the presence of *Hibiscus sabdariffa*, the drug was interacted with the prepared amount 1 g/L of *Hibiscus sabdariffa* extracts in each dissolution media (900 mL). The process described above was then repeated to determine the drug content after each sampling.

Statistical analysis

The results obtained were analysed using Student's T-test with GraphPad Prism 6 software. P values less than 0.05 were considered to be statistically significant.

RESULTS

The results of the identification test for both lisinopril reference standard and tablets revealed the presence of the active ingredient, and the content was within the official limits (98.5 to 101.5 % for standard powder, and 92.5 to 105.0 % for tablet). These indicate that the standard powder and tablet used for this study are genuine and contained the active pharmaceutical ingredients (APIs) in the labelled and stated amount. The results of the *in vitro* availability of lisinopril at different time intervals in simulated gastric juice, intestinal pH and blood pH at 37 ^{O}C alone and in the presence of *Hibiscus sabdariffa* water extract (1g/L) are shown in figures 2 and 3 respectively. It was observed that pH has no influence on the dissolution pattern of lisinopril as shown by the comparable drug release profile in all the media (0.1 M HCl, phosphate buffer pH 6.8 and 7.4) employed in this study.

Paired Student's t-test conducted revealed no statistically significant ($p < 0.05$) difference in the availabilities of lisinopril in the three media (availability of lisinopril in phosphate buffer pH 7.4 taken as the reference). Thus, the lisinopril's release profile in phosphate buffer pH 7.4 was taken as representative for comparison with its availability when interacted with *Hibiscus sabdariffa*.

The WHO requirement for drugs in Class 3 of the BCS, such as lisinopril, is very rapid dissolution profile; specifically, such drugs must release 85% or more of the drug content in 15 min at 75 rpm using multiple time points (WHO, 2006). This requirement differs from the USP and BP (USP, 2012; BP, 2013), which specifies an 80 % release in 30 min (single time point) using the paddle at 50 rpm and 75 % release in 30 min using the paddle at 100 rpm. This study used 50 rpm, 900 mL of dissolution media volume and the BP standard of ≥ 85 % drug release in 30 minutes was considered which the drug satisfied (BP, 2013).

Results of the *in vitro* dissolution test showed that lisinopril achieved a maximum availability of 89.40 and 92.62 % in simulated gastric pH for lisinopril alone and in the presence of *Hibiscus sabdariffa*, respectively. The corresponding contents of lisinopril observed in simulated intestinal pH were 89.40 and 92.51 %, while in simulated blood, pH 89.40 and 91.95 % of lisinopril were released. The presence of *Hibiscus sabdariffa* significantly ($p < 0.05$) increased

the *in vitro* availability of lisinopril in all the three simulated biological pH media (Figure 3). These increases in availability of lisinopril can therefore be attributed to *Hibiscus sabdariffa*, that may have influenced the dissolution of the drug and its subsequent release; which was not observed in the dissolution of lisinopril alone in all the media (Figure 2). Obamiro *et al.* (2013) reported a significant increase in the dissolution profile of lisinopril in simulated pH 4.5 and 6.8 when co-administered with black tea, while a significant decrease in the release profile was observed at pH 1.2. Thus, they recommended that coadministration of black tea with lisinopril should be discouraged as it can impact on tablet dissolution which may result in unpredictable effects. Akinleye *et al.* (2007) reported a significant decrease in the dissolution profile of ciprofloxacin when interacted with five alive juice® in 0.1N HCl, they suggested that to avoid drug therapeutic failures and subsequent bacterial resistance as a result of subtherapeutic level of the drug in the systemic circulation, ingestion of the juice with ciprofloxacin should be discouraged. Olubunmi*et al.* (2015) also reported that Yoyo Bitters (a liquid oral herbal medication reported to be beneficial in the management of illnesses such as diabetes, hypertension, obesity, and for the general well-being of the body) significantly increased the dissolution of lisinopril at pH 1.2 while it was significantly decreased at pH 6.8. However, its dissolution was not significantly affected by Yoyo Bitters at pH 4.5. The significant increase in the availability of lisinopril when it was interacted with *Hibiscus sabdariffa* in simulated gastric, intestinal and blood pH observed in this study could enhance the dissolution of lisinopril when interacted or administered with *Hibiscus sabdariffa* especially in the stomach (as observed by significant increase in the drug availability in simulated gastric pH 1.2) and the drug subsequent absorption in the blood (also as observed in simulated blood pH 7.4) all of which are statistically significant ($p < 0.05$). Studies above have shown that black tea, fruit juice and other herbal supplement have effect(s) (increase or decrease) on the dissolution profile of drugs most of which are statistically significant. This study also shows similar trend. However, the clinical implication of these observations will have to be further validated through *in vivo* pharmacokinetic interaction of lisinopril with *Hibiscus sabdariffa* since it was reported by Allison *et al.* and Hopkins *et al.* (2013) to possess antihypertensive properties as with the drug in question to address the possibility of their co-administration *in vivo*.

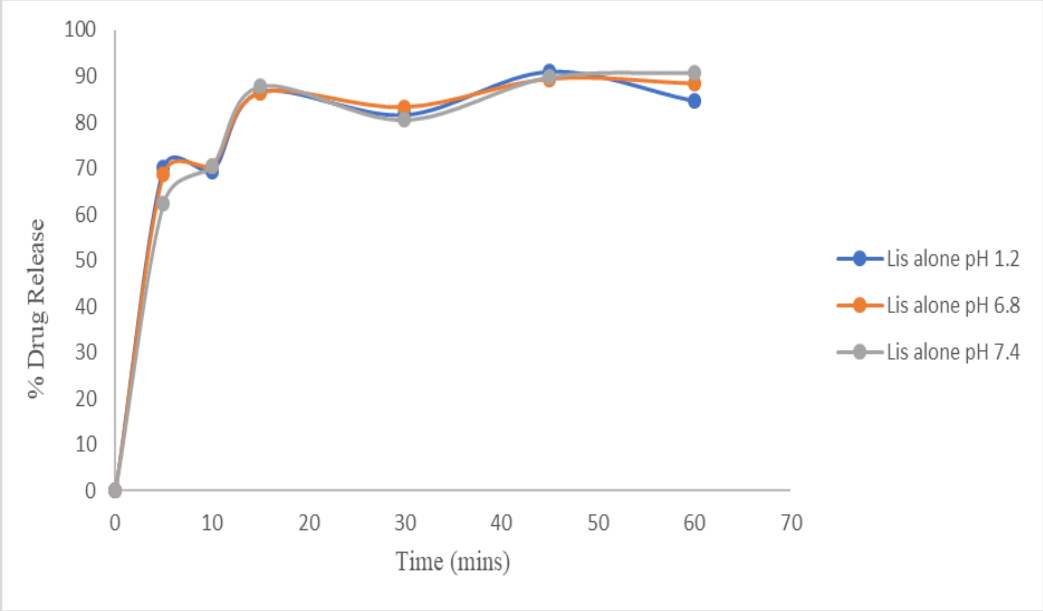

Figure 2: Dissolution profile of lisinopril alone in simulated gastric (pH 1.2), intestinal (pH 6.8) and blood (pH 7.4) media

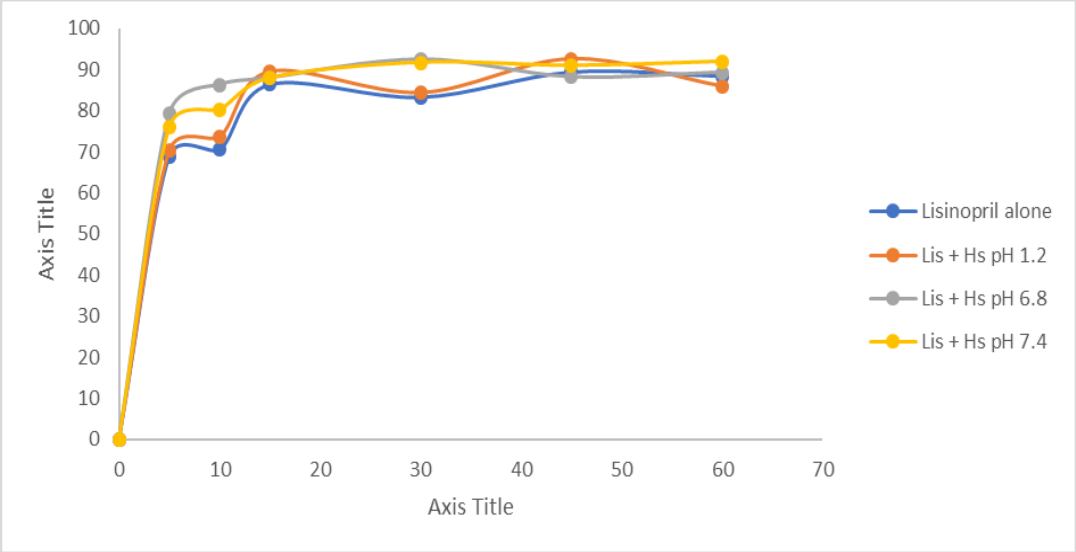

Figure 3: Dissolution profile of lisinopril alone and when interacted with *Hibiscus sabdariffa*in simulated gastric (pH 1.2), intestinal (pH 6.8) and blood (pH 7.4) media

CONCLUSION

We conclude that coadministration of lisinopril with *Hibiscus sabdariffa* calyxes water extracts could enhance its *in vitro* availability consequent to the increased dissolution of lisinopril at simulated gastric, intestinal and blood pH. Further studies on the effect of varying concentrations of *Hibiscus sabdariffa* (calyxes) water extracts on the bioavailability of lisinopril *in vivo* should be evaluated.

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