Effect of Hibiscus Sabdariffa (Linn) Water Extract on the Pharmacokinetics of Lisinopril in Healthy Human Volunteers

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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 Hibiscus sabdariffa and drugs for the treatment of various ailments is a common practice amongst patients; a practice that could result in drug–herb interaction.

Objective: To evaluate the effects of Hibiscus sabdariffa water extract on the pharmacokinetics of lisinopril.

Methodology: The study involved 24 apparently healthy humans randomly selected and divided into two groups of 12 each. Study was carried out in four phases as crossover study with a washout period of two weeks between the phases; Group 1 (received lisinopril 10 mg alone), group 2 (received lisinopril 10 mg with 200 mL Hibiscus sabdariffa calyxes water extract (25 mg/mL) concurrently), group 2 (received lisinopril 30 min after administration of Hibiscus sabdariffa water extract) and group 1 (received Hibiscus sabdariffa water extract 30 minutes after administration of lisinopril). Saliva samples collected at 0.5 to 36.0 hr in each phase were analysed for lisinopril content using a developed and validated RP-HPLC method. Results were analysed using one-way Anova followed by Tukey’s post Hoc.

Results: Lisinopril administered alone (group 1) achieved Cmax of 95.36 ng/mL at 7 hr (Tmax). Slight increases in Cmax of lisinopril were observed when the drug was interacted with Hibiscus sabdariffa in groups 2 (97.50 ng/mL), 3 (97.37 ng/mL) and 4 (99.24 ng/mL) respectively. The (AUC0∞) of lisinopril alone was 736805.83 ng.hr/mL. The corresponding AUC0∞ for group 2, 3 and 4 are 800409.29, 816866.47, and 10450.03 ng.hr/mL respectively. However, these increases in Cmax and AUC0∞ seen were not statistically significant (p < 0.05). Pharmacokinetic parameters were computed using Kinetica software.

Conclusion: Hibiscus sabdariffa calyxes water extract (200 mL of 25 mg/mL) does not significantly affect the pharmacokinetics of lisinopril in healthy humans.

Keywords: Lisinopril; Hibiscus sabdariffa; Pharmacokinetics; Human volunteers
INTRODUCTION

Lisinopril (figure1) is a metallopeptidase antihypertensive drug belongs to angiotensin converting enzyme inhibitor (BP, 2013). Lisinopril is already an active diacid and does not need to be metabolised in vivo. Peak concentrations in plasma are reported to occur after about 7 hr. Lisinopril is reported not to be significantly bound to plasma proteins, this is reflected by its high volume of distribution and is excreted unchanged in the urine (Sweetman, 2002).

Herbal medicines are accepted and commonly used worldwide with a hope of improving health conditions and managing various diseases such as pains, cold, inflammation, diabetes and heart diseases. However, their mechanisms of action are generally unknown and there is a lack of clinical efficacy and safety data (Sahoo et al., 2010). An estimated one fourth of adults in developed countries and more than 80% of the population in most developing countries utilize herbal medicines (Mehta et al., 2008). African traditional medicine in Nigeria has survived through the worst periods of human existence, the period of slave trade (Spitzer, 2002). This is shown in the level of dependence of Nigerians from local areas in the twenty-first century on the traditional medicinal system which has outlived several other systems (Carney, 2003; Mitchell and Ahmad, 2006). During the period of slave trade, slaves from West Africa particularly Nigeria, usually predominate and emerge as the traditional healers of the African settlements (Carney, 2003). A study conducted which covered 66.6% of Edo state, central southern Nigeria, reported 70 plants belonging to 67 genera in 43 families are commonly prescribed, these herbal antihyperglycaemic and antihypertensive were prepared as decoctions, infusions, powders and or juice before use (Gbolade, 2012). Another reported study carried out in Sokoto State located in north west Nigeria reported the interview of 40 traditional medicine practitioners and herbal sellers (65% males and 35% females) using semi-structured questionnaires indicated 34 of the plants belonging to 30 families with reports of validation of some of the antihypertensive and antihyperglycaemic potential of the plants some of which include Allium sativum, Commiphora kastingii, Moringa oleifera and Hibiscus sabdariffa (Raji et al., 2013). Decoctions of Hibiscus sabdariffa have been used locally in West Africa and Mexico in the management of high blood pressure. Several in vitro and in vivo studies (Obiefuna et al., 1994) have shown that the extract of the calyces of Hibiscus sabdariffa (in the range 125 to 500 mg/kg) greatly reduce both the systolic and diastolic blood pressures, lowering heart rate and acts as a vasodilator (Inuwa et al., 2012). The blood pressure lowering activity might be through inhibition of angiotensin-converting enzymes (ACE) (Ojeda et al., 2010), acetylcholine-like and histamine-like mechanisms (Adegunloye et al., 1996), diuretic effect (Mojiminiyi et al., 2000), reduction in the diffusion distance between capillaries and myocytes, as well as new vessel formation mechanisms (Inuwa et al., 2012) and direct relaxation of blood vessels (Adegunloye et al., 1996 and Ajay et al., 2007). Moreso, Hibiscus sabdariffa showed antplatelet but no thrombolytic activity in vitro (Yamamoto et al., 2005). Despite the beneficial effect of Hibiscus sabdariffa as an antihypertensive, it was reported to produce an unwanted effect on gonads (Da-Costa-Rocha et al., 2014). There are reports of several clinical trials that were carried out to determine the blood lowering effect of Hibiscus sabdariffa (Herrera-Arellano et al., 2004; Herrera-Arellano et al., 2007; Mozaffari-Khosravi et al., 2009). A systematic review carried out in 2010 concluded that the studies did not provide reliable evidence to support recommendation of Hibiscus sabdariffa plant to control or lower blood pressure in hypertensive patients, when compared to placebo or no treatment used (Ngamjarus et al., 2010; Wahabi et al., 2010). However, in a randomised, double-blind, placebo-controlled clinical trial, the results showed that H. sabdariffa tea (1.25 g of H. sabdariffa per 240 mL in boiled water) effectively reduced blood pressure in pre-and mildly hypertensive adults involved in the study (McKay et al., 2010). Similar effects on decreasing systolic and diastolic blood pressures were observed in moderately hypertensive Type 2 diabetic (comorbid) individuals when taking green or hibiscus tea for one month (Mozaffari-Khosravi et al., 2013). Furthermore, another comprehensive review on lower animal and human studies on the effect of Hibiscus sabdariffa in the treatment of hypertension and hyperlipidemia concluded that Hibiscus sabdariffa has great potential to reduce risk factors associated with cardiovascular diseases (Hopkins et al., 2013).
Secondary metabolites like anthocyanins, including delphinidin-3-O-sambubioside (hibiscin) and cyanidin-3-O-sambubioside (gossypicyanin), have been identified as being responsible for angiotensin converting enzyme inhibition of *Hibiscus sabdariffa* (Herrera-Arellano et al., 2007; Ojeda et al., 2010).

It is evident that herbal remedies are often being taken together with orthodox drugs, raising the potential for herb-drug interactions, which may have important clinical significance based on an increasing number of clinical reports of such interactions reported (Chen et al., 2012; Babos et al., 2021). The interaction of herbal medicines with prescribed drugs is a significant safety concern, especially for drugs with narrow therapeutic indices because the pharmacokinetics and/or pharmacodynamics of the drug may be altered by combination with the herbal remedies, severe and perhaps even life-threatening adverse reactions may occur in clinical practice. Due to the clinical significance of herbal interactions with conventional drugs, it is important to identify herbs and drugs that may interact with each other and cause clinical consequences (Chen et al., 2012; Babos et al., 2021). It has been recommended that, particularly for drug action that is potentiated by the same effect of herbs addition of herbal therapy should be avoided when the drug therapy is already addressing the therapeutic goal and only a well-reasoned analysis of the potential for drug herb interaction will often permit their use together (Subhuti, 2000).

This research is aimed at evaluating the effect of *Hibiscus sabdariffa* Linn water extract on the pharmacokinetics of lisinopril in healthy human volunteers.

**Figure 1: Chemical structure of lisinopril**

**METHODOLOGY**

**Equipment and Reagents**

Caffeine standard powder, HPLC grade methanol and water obtained from Sigma Aldrich (Germany). HPLC column: Chemsl ODS C18 (200 mm×4.6 mm i.d., 5µ particle size), Shimadzu D439300179 digital analytical weighing balance, Thermo Electron Corporation Centra CL2 centrifuge, HPLC sample bottles 1.5 mL, HPLC machine used was Agilent technologies (Model 1200 Infinity Series). FTIR machine (Agilent technologies model 1260 Infinity Series). A double scanning UV/vis spectrophotometer (Model SP 3000) was also used.

The plant *Hibiscus sabdariffa* was collected in Samaru, Zaria during the month of September, 2016. It was identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria by Malam Namadi Sanusi and was assigned a voucher number of 1056. The calyces were removed, shed dried and size reduced and kept in air tight container for subsequent use. Lisinopril (Zestril brand 10 mg) was purchased from a reputable pharmacy in Samaru, Zaria, Nigeria.

**Preparation of Solutions**

**Preparation of suitable solvent for the drug and internal standard (IS)**

Although lisinopril is highly soluble in water, it was observed that the solvent that gives better resolution both for the drug and internal standard (caffeine) is methanol:water (M:W) in a ratio 50:50 v/v. This solvent was used in dissolving the lisinopril and IS throughout the analysis (Nasir et al., 2021).

**Preparation of stock solution of the caffeine (Internal Standard)**
A stock solution (100 µg/mL) of caffeine was prepared by accurately weighing and dissolving 4 mg of caffeine powder in 40 mL of methanol:water (M:W).

**Study design and inclusion criteria**

The total number of subjects that participated were 24 who were randomly selected and divided into two (2) groups of 12 subjects each. Study was carried out in four (4) phases as double-blind simple randomized crossover studies with a washout period of two (2) weeks in between the phases.

This study was conducted using the volunteers who satisfied the following inclusion and exclusion criteria. Inclusion criteria included healthy male volunteers, aged between 18 and 40 years with body mass index (BMI) in the range of 18 – 25 kg/m$^2$. Persons with any of the following characteristics were excluded from the study: history of smoking (more than 10 cigarettes per day), history of regular alcohol consumption, taking other medications within two (2) weeks before entering the study, allergic reactions and hereditary disorder (angioedema) in which lisinopril is contraindicated, subjects with history of liver, kidney and cardiovascular disease that could affect bioavailability of lisinopril, subjects that participated in other clinical experiments within 1 month prior to this study, subjects that took lisinopril within the previous two weeks or are currently taking lisinopril.

**Preparation and standardization of Hibiscus sabdariffa water extract**

The *Hibiscus sabdariffa* solution in water was prepared by weighing a quantity (5) g of *Hibiscus sabdariffa* calyxes and cold macerated in 1 L of water for 24 hr. It was filtered and a portion of the filtrate (100 mL) was evaporated to dryness using water bath at 65°C. The extract was weighed and the concentration was found to be 0.25 g/100 mL of the extract. By diluting 100 mL of the filtrate containing 0.25 g/100 mL with water to 1 L a solution with strength of 25 mg/mL was obtained.

**Phase 1 (Administration of drugs alone)**

Blank saliva was collected from each subject in group 1 before administration of lisinopril tablet (10 mg) with 200 mL water. Thereafter, saliva samples were collected at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 12.0, 24.0 and 36.0 hr. A quantity (2 mL) of saliva samples for each volunteer was treated as described by Nasir et al. (2021) as follows:

The prepared suitable solvent consisting of M:W (2 mL each) were transferred into series of 5 mL sample bottles each containing the collected saliva (2 mL) samples followed by 1 mL of a solution (0.05 µg/mL) of the internal standard caffeine. The mixtures were vortex mixed and centrifuged at 3000 rpm for 10 min resulting in clear solutions. A quantity (0.5 mL) of each clear solution was injected into the HPLC machine and the respective chromatograms were obtained. The corresponding concentrations of peak height ratios of lisinopril/internal standard obtained were then interpolated from the calibration curve (Nasir et al., 2021).

**Phase 2 (Concurrent administration of lisinopril with Hibiscus sabdariffa water extracts)**

In this phase blank saliva was collected from each subject in group 2 before administration of lisinopril tablet (10 mg) concurrently with 200 mL of *Hibiscus sabdariffa* water extracts (25 mg/mL). Thereafter, saliva samples were collected at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 12.0, 24.0 and 36.0 hr. A quantity (2 mL) of saliva samples for each volunteer was treated as described by Nasir et al. (2021). The next two phases (phase 3 and 4) were carried out after the washout period of two (2) weeks.

**Phase 3 (Administration of lisinopril 30 min after administration of Hibiscus sabdariffa water extracts)**

In this phase, blank saliva was collected from each subject in group 2 before administration of 200 mL of *Hibiscus sabdariffa* water extracts (25 mg/mL). After 30 min lisinopril tablet (10 mg) was administered to the volunteers and saliva samples were collected at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 12.0, 24.0 and 36.0 hr. A quantity (2 mL) of saliva samples for each volunteer was treated as described in phase 1 and 2 above.

**Phase 4 (Administration of Hibiscus sabdariffa water extracts 30 min after administration of lisinopril)**

In this phase, blank saliva was collected from each subject in group 1 before administration of lisinopril tablet (10 mg). After 30 min 200 mL of *Hibiscus sabdariffa* water extracts (25 mg/mL) was administered the volunteers and saliva samples were
collected at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 12.0, 24.0 and 36.0 hr. A quantity (2 mL) of saliva samples for each volunteer was treated as described in phase 1 and 2 above.

**Evaluation of pharmacokinetic parameters**

The drug-internal standard peak height ratio obtained from the chromatograms of each volunteer were used to determine the drug concentrations by interpolating from the calibration curve of the drug (Nasir et al., 2021). Pharmacokinetic parameters like elimination rate constant ($K_E$), biological half-life ($t_{1/2}$), absorption rate constant ($K_a$), absorption half-life ($t_{1/2}$), volume of distribution ($V_d$), clearance (Cl), maximum plasma drug concentration ($C_{max}$), time at which $C_{max}$ is observed ($T_{max}$) and total area under the curve ($AUC_{0-\infty}$) which is the sum of the last and extrapolated areas under concentration time curve ($AUC_{0}^{d} + AUC_{\infty}^{c}$) were all computed using Kinetica software version 5.0 from the mean drug plasma concentrations.

**Statistical analysis**

Results obtained were analyzed with Graph Pad Prism 6 software using Students $t$-test and one-way ANOVA then followed by Tukey’s Post hoc test where applicable and it was presented as percentages, means ± SD.

**RESULTS**

*In vivo interaction study of metformin and lisinopril with Hibiscus sabdariffa L.*

Mean saliva lisinopril concentrations versus time obtained from the volunteers in all the four phases of the interaction study are displayed in Figure 2 and Table 1. The mean pharmacokinetic parameters generated for lisinopril administered alone and when interacted with *Hibiscus sabdariffa* calyces water extracts in phase 2, 3, and 4 are also displayed in Table 2.

![Figure 2: Mean saliva concentrations of lisinopril Vs time for all the phases of the study](image_url)
Table 1: Mean saliva concentrations versus time for lisinopril (10 mg) alone and when interacted with 200 mL of Hibiscus sabdariffa calyxes water extract (25 mg/mL)

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Phase 1 mean values ± SD (ng/mL)</th>
<th>Phase 2 mean values ± SD (ng/mL)</th>
<th>Phase 3 mean values ± SD (ng/mL)</th>
<th>Phase 4 mean values ± SD (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>11.23 ± 0.81</td>
<td>13.52 ± 0.17</td>
<td>12.87 ± 0.65</td>
<td>15.94 ± 0.38</td>
</tr>
<tr>
<td>2</td>
<td>21.49 ± 0.49</td>
<td>24.63 ± 0.38</td>
<td>24.24 ± 0.52</td>
<td>26.32 ± 0.49</td>
</tr>
<tr>
<td>3</td>
<td>34.24 ± 0.31</td>
<td>38.33 ± 0.31</td>
<td>36.45 ± 0.56</td>
<td>37.64 ± 0.45</td>
</tr>
<tr>
<td>4</td>
<td>52.73 ± 0.37</td>
<td>56.23 ± 0.21</td>
<td>55.35 ± 0.42</td>
<td>56.85 ± 0.85</td>
</tr>
<tr>
<td>5</td>
<td>71.66 ± 0.90</td>
<td>73.34 ± 0.43</td>
<td>72.50 ± 0.74</td>
<td>74.34 ± 0.56</td>
</tr>
<tr>
<td>6</td>
<td>85.46 ± 0.46</td>
<td>87.52 ± 0.23</td>
<td>87.55 ± 0.79</td>
<td>88.64 ± 0.73</td>
</tr>
<tr>
<td>7</td>
<td>95.36 ± 0.39</td>
<td>97.50 ± 0.09</td>
<td>97.37 ± 0.50</td>
<td>99.24 ± 0.86</td>
</tr>
<tr>
<td>8</td>
<td>80.58 ± 0.46</td>
<td>83.55 ± 0.57</td>
<td>82.46 ± 0.47</td>
<td>80.36 ± 0.37</td>
</tr>
<tr>
<td>9</td>
<td>62.27 ± 0.25</td>
<td>65.31 ± 0.37</td>
<td>64.68 ± 0.79</td>
<td>65.12 ± 0.57</td>
</tr>
<tr>
<td>12</td>
<td>30.59 ± 0.65</td>
<td>33.29 ± 0.39</td>
<td>35.49 ± 1.46</td>
<td>50.07 ± 0.63</td>
</tr>
<tr>
<td>24</td>
<td>1.53 ± 0.07</td>
<td>2.52 ± 0.07</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>36</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Number of participants = 12 for each phase; ND = not detected

Table 2: Mean pharmacokinetic parameters of lisinopril (10 mg) alone and when interacted with 200 mL of Hibiscus sabdariffa calyxes water extract (25 mg/mL)

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tlag (hr)</td>
<td>0.500</td>
<td>0.500</td>
<td>0.500</td>
<td>0.500</td>
</tr>
<tr>
<td>K_a (ng hr⁻¹)</td>
<td>0.557</td>
<td>0.601</td>
<td>0.633</td>
<td>0.915</td>
</tr>
<tr>
<td>K_e (ng hr⁻¹)</td>
<td>0.248</td>
<td>0.216</td>
<td>0.204</td>
<td>0.132</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>2.799</td>
<td>3.202</td>
<td>3.391</td>
<td>5.270</td>
</tr>
<tr>
<td>C_max (ng mL⁻¹)</td>
<td>95.360</td>
<td>97.500</td>
<td>97.370</td>
<td>99.240</td>
</tr>
<tr>
<td>T_max (hr)</td>
<td>7.000</td>
<td>7.000</td>
<td>7.000</td>
<td>7.000</td>
</tr>
<tr>
<td>AUC₀→∞ (ng.hr mL⁻¹)</td>
<td>730602.846</td>
<td>788798.425</td>
<td>643247.706</td>
<td>679.104</td>
</tr>
<tr>
<td>AUC₀→∞ (ng.hr mL⁻¹)</td>
<td>6202.982</td>
<td>11610.866</td>
<td>173618.763</td>
<td>365.923</td>
</tr>
<tr>
<td>Cl (L/hr⁻¹)</td>
<td>13.572</td>
<td>12.494</td>
<td>12.242</td>
<td>9.569</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>54.802</td>
<td>57.721</td>
<td>59.892</td>
<td>72.756</td>
</tr>
</tbody>
</table>

DISCUSSION

The results of the in vivo availability study (Figure 2 and Table 1) showed significant increase (p < 0.05) in the availabilities of lisinopril when interacted with Hibiscus sabdariffa in all the phases of interaction study which is in agreement with the in vitro availability study findings of the drug (Nasir et al., 2019).

The pharmacokinetic study results showed that lisinopril administered alone achieved C_max of 95.360 ng/mL at 7 hr (T_max). Slight increases in C_max of lisinopril were observed when the drug was interacted with Hibiscus sabdariffa in phase 2 (97.500 ng/mL), phase 3 (97.370 ng/mL) and phase 4 (99.240 ng/mL). However, these increases were not statistically significant (p < 0.05). Peak concentrations in plasma are reported to occur after about 7 hr (Sweetman, 2002). Olcay and Lale (2004) reported that lisinopril administered alone in healthy volunteers attained C_max of 87.4 ng/mL at 7 hr. This is comparable to the observed C_max and T_max of lisinopril in phase 1 of this study.
Total area under the concentration time curve ($AUC_0^\infty$) of lisinopril administered alone was 736805.828 ng.hr/mL. The corresponding $AUC_0^\infty$ for phase 2, 3 and 4 are 800409.291, 816866.469, and 1045.027 ng.hr/mL respectively. However, these increases in $C_{\text{max}}$ and $AUC_0^\infty$ also seen with lisinopril were not statistically significant ($p < 0.05$). On the other hand, a decrease in clearance (Cl) of lisinopril from 13.57 L/hr in phase 1 to 9.57 L/hr in phase 4 was observed, this indicate an increase in the mean residence time of the drug, however, the observed decrease is not statistically significant ($p < 0.05$).

Lisinopril is reported not to be significantly bound to plasma proteins this is reflected by its high volume of distribution (Sweetman, 2002). In this study also, a slight increase in volume of distribution of lisinopril was observed in phase 4 (Table 2). However, these decreases are also not statistically significant ($p < 0.05$). These observations, may thus not be clinically significant as lisinopril do not fall among the class of drugs with narrow therapeutic index except in subjects with renal impairment because the excretion of the drug is renal and thus, the drug is contraindicated in them (Sweetman, 2002). Although statistical analysis conducted for all the pharmacokinetic parameters of lisinopril revealed no statistically significant difference ($p < 0.05$), however, the clinical relevance of these findings will have to be validated through further pharmacodynamic interactions of Hibiscus sabdariffa with the drug.

CONCLUSION
It could be concluded that Hibiscus sabdariffa water extract (at 200 mL of 25 mg/mL) does not significantly affect ($p < 0.05$) the pharmacokinetic of lisinopril in healthy humans. However, small increases were observed in the pharmacokinetic parameters; the clinical relevance of which will have to be validated.

Lisinopril can be taken with Hibiscus sabdariffa L. water extract (200 mL of 25 mg/mL) without any fear of their pharmacokinetics been affected. The interactions between Hibiscus sabdariffa and the drugs should be pharmacodynamically evaluated to establish benefit of concurrent use.

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ETHICAL CONSIDERATIONS
Ethical clearance (ABUCUHSR/2017/003) was obtained from the Ahmadu Bello University, Zaria ethical Committee for the Use of Human subjects for Research (ABUCUHSR). An informed written consent was also obtained from the volunteers.

REFERENCES


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