EFFECT OF TWO POLYHERBAL BITTERS ON THE PHARMACOKINETICS OF GLIBENCLAMIDE AND METFORMIN IN RATS

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ABSTRACT
Polyherbal bitters are herbal products which are commonly used by diabetic patients, because they are claimed to have antidiabetic potentials. Promoters of the bitters claim that being a natural product it could be co-administered with other therapeutic drugs with no adverse effects. This research was aimed at assessing the effect(s) or otherwise of the co-administration of two popular polyherbal bitters in Nigeria market (S-bitter SB and Y-bitter YB) on the pharmacokinetics of two widely used antidiabetic drug glibenclamide (GLI) and metformin (MET) using rats. Two hundred and eighty seven rats of both sexes weighing between 180g and 240g were divided into eight groups of thirty five each and the ninth group control with seven. Therapeutic doses of the bitters alone and in combination with glibenclamide and metformin corresponding to the body weight of the animals were administered orally to different groups. At intervals of 0.5, 1, 3, 6, 8, 12 and 24 hours, five rats from each group were sacrificed and blood collected by cardiac puncture. The blood was centrifuged and the plasma collected was analyzed using UV visible spectrophotometer for the concentrations of metformin and glibenclamide respectively. The results obtained indicated that co-administration of metformin with SB decreased $k_a$ from 0.578 to 0.349$h^{-1}$, increased $T_{max}$ from 1.0 to 6.0 hours, but had no significant effect on AUC, $C_{max}$ and $t_{1/2}$. Co-administration
of metformin with YB cause on increase in $t_{\frac{1}{2}}$ from 4 to 6.2 hours, AUC from 12.55 to 18.086 $\mu$ g/h/mL and $T_{\text{max}}$ from 1.0 to 6.0 hours. There was also a decrease in $K_{\text{el}}$ from 0.173 to 0.111 h$^{-1}$ but with no effect on $C_{\text{max}}$. Co-administration of glibenclamide with SB increases $T_{\text{max}}$ from 3 to 6 hours $C_{\text{max}}$ from 200 to 250 $\mu$g/ml, $t_{\text{1/2el}}$ from 9.6 to 14.5 hours and a decrease in $k_{\text{el}}$ from 0.0721 to 0.0477086 $\mu$g/h/mL. Co-administration glibenclamide with YB had no significant effect on any of the pharmacokinetic parameters measured. We concluded that it is not advisable to administer any of the bitters with therapeutic drugs since the effect on their pharmacokinetic parameters is erratic.

**KEYWORDS:** Polyherbal bitters, Pharmacokinetics, Glibenclamide, Metformin.

**INTRODUCTION**

Diabetes mellitus is a complex chronic illness requiring continuous medicinal care. As of 2013, 382 million people had diabetes worldwide.$^{[1,2,3,4]}$ The bitters are galenical oral preparations made from a blend of various parts and fruits of plants. Manufacturers of the various bitters claim that, they cure a wide variety of ailments among which are kidney and bladder infections, normalize interestinal motility, lower blood sugar, act as digestive aid and detoxify the body. Some researchers agree that some have potent antidiabetic effects.$^{[5]}$

A drug must be present in appropriate concentrations at its sites of action to produce its desired effects.$^{[6,7,8]}$ For orally administered drugs, it must be absorbed, from the gastrointestinal tract to an extent and at a rate that will ensure adequate blood levels to elicit pharmacological response of desired magnitude and duration.$^{[8]}$ The efficiency with which a drug is absorbed is a function of many variables among which is drug-drug interaction which has been described for many drugs.$^{[9,10,11,12]}$

The bitters are widely used for chemotherapy in Nigeria. Components of the bitters are known to interact with and influence blood glucose levels.$^{[5]}$ Since consumers of these bitters may do so concurrently with some therapeutic drugs, this study therefore, seeks to establish if there is any significant interaction between two brands of widely used bitters in Nigeria SB and YB and two popular therapeutic antidiabetic drugs GLI and MET. The results obtain will be used to properly advise consumers of these products.
MATERIALS AND METHODS

Ethical approval
Permission and approval for animal studies were obtained from the College of Health Science, Animal Ethics Committee, University of Uyo.

Chemicals
All chemicals used were of analytical grade. Pure glibenclamide and metformin were gift from May and Baker Nigeria Plc. Lagos Nigeria. Pure chloroform and absolute ethanol were purchased from sigma Aldrich. Glibenclamide and metformin tablets and the bitters SB and YB which were NAFDAC registered and less than one year from the date of manufacture were purchased from registered Pharmacy in Uyo. Distilled water was used for all dilution and analysis. A cecil spectrophotometer model number CE 7200 was used for the analysis.

Animals
A total of 287 healthy albino rats of both sexes (Wister strain) weighting between 180 - 240 ± 21g were used in the study. They were maintained under standard environmental condition and had free access to food and water at the animal house, University of Uyo.

Preparation of standard calibration curve for glibenclamide and metformin
10mg of pure glibenclamide was dissolved in 10ml of chloroform and 10mg of pure metformin was dissolved in 10ml of ethanol. Aliquots of 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml, 3.5ml, 4.0ml, 4.5ml and 5.0ml of the stock solutions were measured into 10ml volumetric flask and appropriate volumes of chloroform and ethanol respectively were added to make up 10.0ml. The absorbance of glibenclamide was measured at 260nm against chloroform as blank while that of metformin was measured at 320nm against ethanol as blank. A calibration graph of concentration against absorbance was plotted for glibenclamide and metformin respectively.[13]

Administration of Test Materials
The rats were fasted overnight prior to the testing. They were divided into 8 groups; A, B, C, D, E, F, G and H. Comprising 35 rats each. The various combinations administered are shown in table 1. The ninth group I contained seven rats and one was sacrificed at each interval and their plasma used as blank.
At intervals of 0.5 hours, 1.0 hours, 3.0 hours, 6 hours, 8 hours, 12.0 hours and 24.0 hours, blood was collected from five rats in each group by cardiac puncture under chloroform anesthesia into EDTA tubes.[14]

Table 1: Administration of Test Materials

<table>
<thead>
<tr>
<th>Group</th>
<th>Material Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glibenclamide alone 5mg/kg body weight.</td>
</tr>
<tr>
<td>B</td>
<td>Glibenclamide 5mg/kg + S-bitters 15ml/kg.</td>
</tr>
<tr>
<td>C</td>
<td>Glibenclamide 5mg/kg + Y-bitters 20ml/kg.</td>
</tr>
<tr>
<td>D</td>
<td>Glibenclamide 5kg/kg + S-bitters 15ml/kg + Y-bitters 20ml/kg</td>
</tr>
<tr>
<td>E</td>
<td>Metformine alone 15mg/kg</td>
</tr>
<tr>
<td>F</td>
<td>Metformin 15mg/kg + S-bitters 15ml 1kg</td>
</tr>
<tr>
<td>G</td>
<td>Metformin 15mg/kg + Y-bitters 20ml/kg</td>
</tr>
<tr>
<td>H</td>
<td>Metformin 15mg/kg + S-bitters 15ml/kg + Y-bitters 20l/kg</td>
</tr>
<tr>
<td>I</td>
<td>Normal saline only</td>
</tr>
</tbody>
</table>

The whole blood collected was allowed to stand for 10 minutes to cool and equilibrate with the anticoagulant. The blood was then centrifuged for 30 minutes at 5000gpm. The plasma was aspirated into sterile sample tubes. 2.0ml of plasma collected was diluted with 1.0ml of distilled water and the absorbance was measured at 260nm for glibenclamide and 310nm for metformin using blank diluted plasma as reference. The concentration of the drugs in plasma was extrapolated from the various calibration curves earlier prepared.

Statistical analysis

Data were expressed as mean ± SEM. Statistical comparisons between groups were performed using analysis of variance (ANOVA). Differences between means were determined by Tukey-Kramer pair-wise comparison test at a level of significance p < 0.05.

RESULTS AND DISCUSSION

Maximum serum concentration (C_{max}) and time required to attaining C_{max}, \( t_{max} \) were obtained direct from the serum concentration-time graph. From the results of these studies, these parameters agree with those on the drug literature when both drugs are administered alone, however, there was a significant increase in C_{max} and \( t_{max} \) p<0.05 when glibenclamide was concurrently administered with SB and with SB and YB, but there was no effect on C_{max} when YB was administered with the same drug (table 2). When metformin was concurrently administered with SB, YB and SB + YB, there was no significant effect on C_{max} (Table 3). For many drugs a relationship exists between the pharmacologic effect of a drug and the plasma drug concentration. C_{max} also provides an indication that the drug is sufficiently
absorbed systemically to provide the required therapeutic response, it also provides warning of possible toxic levels of drugs. The increase in $C_{\text{max}}$ may be as a result of suppression of metabolizing enzymes of glibenclamide. This increase in $C_{\text{max}}$ may result in toxicity when glibenclamide is co-administered with SB.[15,16]

The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule as described by Shargel and Yu (1984). The AUC is a measurement of the extent of bioavailability. It reflects the amount of active drug which reaches systemic circulation to elicit pharmacological effect. The result of this study showed a significant increase in the AUC when glibenclamide is co-administered with both SB and YB (Table 2). However AUC was affected significantly only when metformin was co-administered with YB, but with SB there was no effect in AUC. AUC could not be determined when metformin was co-administered with both SB and YB because $C_{\text{max}}$ and the curve was not completed within the 24.0 hour period of the experiment. The results shows that the co-administration of some bitters with therapeutic drugs resulted in an increase in bioavailability as shown in increase in AUC, in other cases there were no effect on AUC while in others the drug remained in circulation for a very long time, these could lead to toxicity or hypoglycaemia.[17] Worst still the results are not consistent with all the bitters and the therapeutic drugs tested.

The time to attain maximum plasma concentration $t_{\text{max}}$ increased significantly $p < 0.05$ when GLI was co-administered with SB but no effect with YB. SB and YB respectively caused a significant increase in $t_{\text{max}}$ when used concurrently with MET (Table 3). The increase in $t_{\text{max}}$ may cause a delay in the on-set of therapeutic action.[17,18] This might lead to treatment failures and serious consequence on the patience especially during emergency.[18,19,20]

Absorption rate constant, $k_a$ is given by first order rate equation:

$$\log C_1 = -K_t/2.303 + \log C_0.$$  

The graph of the log of concentration against time gave a straight line with the slope as $K_a$. The results of this study showed a significant decrease in $K_a$, $p < 0.05$ when GLI is co-administered with SB and MET is co-administered with SB and YB respectively.

Factors such as surface area of the gut, stomach emptying rate, gastrointestinal motility, and blood flow to the absorption site all affect the rate and extent of drug absorption. Reduction
in $K_a$ by some bitters may be as a result of physicochemical reactions such as chelation and adsorption of the drugs by components of the bitters. The same reason could be responsible for the decrease in $C_{\text{max}}$ and increase in $t_{\text{max}}$.\textsuperscript{[9,10,18]}

The elimination rate constant $k_{\text{el}}$ was evaluated from the elimination phase of the serum concentration-time graph on a semi-log paper. Elimination of drugs proceeds by excretion or biotransformation or both. The decreased $k_{\text{el}}$ as observed in co-administration of GLI with SB and MET with YB could be due to suppression in the production of metabolizing enzymes by some components of the bitters.\textsuperscript{[15]}

Elimination half life $T_{1/2}$ el. is the time required for half the total drug in circulation to be eliminated. It was calculated from the formula $t_{1/2} \text{ el} = 0.693/k_{\text{el}}$.

The effect of the bitters on $t_{1/2}$ el is as shown in tables 2 and 3.

Table 2: Pharmacokinetic parameters of glibenclamide (GLI) alone and in combination with S-bitter (SB) and Y-bitter (YB).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GLI Alone</th>
<th>GLI + SB</th>
<th>GLI + YB</th>
<th>GLI + SB + YB</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>2001.0 ± 144</td>
<td>2503.1±321.51</td>
<td>2008.3±391.4</td>
<td>2607.0±567.02</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>3.0 ± 0.47</td>
<td>6.0±0.72</td>
<td>3.0±0.72</td>
<td>6.0±0.87</td>
</tr>
<tr>
<td>AUC(μg/h/mL)</td>
<td>2570 ± 392</td>
<td>2968±431</td>
<td>2792±399</td>
<td>4313±573</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.0313 ± 0.004</td>
<td>0.0201±0.0037</td>
<td>0.0333±0.0021</td>
<td>0.025±0.0011</td>
</tr>
<tr>
<td>$k_{\text{el}}$(h$^{-1}$)</td>
<td>0.0721±0.0037</td>
<td>0.0477±0.0051</td>
<td>0.0767±0.0017</td>
<td>0.0576±0.0077</td>
</tr>
<tr>
<td>$t_{1/2}$ el (h)</td>
<td>9.60 ± 0.93</td>
<td>14.5±1.03</td>
<td>9.04±0.89</td>
<td>12.03±1.07</td>
</tr>
</tbody>
</table>

Mean ± SD, n=5, *p<0.05

Table 3: Pharmacokinetic parameters of metformin (MET) alone and in combination with S-bitter (SB) and Y-bitter (YB)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MET Alone</th>
<th>MET + SB</th>
<th>MET + YB</th>
<th>MET+ SB + YB</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>1600.0±271</td>
<td>1540.0±131</td>
<td>1560.0±102</td>
<td>-</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.0±0.30</td>
<td>6.00±0.83</td>
<td>6.00±0.99</td>
<td>-</td>
</tr>
<tr>
<td>AUC (μg/h/ml)</td>
<td>1255.60±117</td>
<td>1200.20±187</td>
<td>1808.60±103</td>
<td>-</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.578±0.003</td>
<td>0.347±0.003</td>
<td>0.315±103</td>
<td>-</td>
</tr>
<tr>
<td>$k_{\text{el}}$(h$^{-1}$)</td>
<td>0.173±0.003</td>
<td>0.173±0.006</td>
<td>0.111±0.0071</td>
<td>-</td>
</tr>
<tr>
<td>$T_{1/2}$ el (h)</td>
<td>4.00±0.51</td>
<td>4.000±0.55</td>
<td>6.20±0.57</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean ± SD, n=5, p < 0.05 , - could not be determined within hours.

CONCLUSION

Phytochemicals as contained in polyherbel bitters are known to interact with drug transporters thereby causing impairment or exaggeration of pharmacological activity.\textsuperscript{[10]}
Some elements, notably zinc, may induce intestinal proteins which bind drugs and prevent their transfer from the intestine into the body.[11] This study has shown that the co-administration of the polyherbal bitters with therapeutic glibenclamide and metformin resulted in varying pharmacokinetic parameters, which could lead to delay in therapeutic action, therapeutic failure, and prolonged pharmacologic activity resulting in organ toxicity. It was therefore concluded that, since the polyherbal bitters cause erratic changes in pharmacokinetic parameters of glibenclamide and metformin, they should not be administered concurrently to avoid unwanted effects.

ACKNOWLEDGEMENT
The authors are grateful to Mr. Nsikan Malachy, laboratory technician, of the animal house of the department of Pharmacology and Toxicology, University of Uyo, and Mrs. Ekaete Umoh, the Chief Technologist of the Department of Pharmaceutical and Medicinal Chemistry, University of Uyo for their Technical Assistance.

REFERENCES