Comparison of the effects of cimetidine and hyoscine-n-butyl bromide on paracetamol pharmacokinetics in healthy human volunteers

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ABSTRACT

Cimetidine is a H_2-receptor antagonist used in the management of peptic ulcer and other acid hypersecretory conditions. It was previously thought to have no effect on gastric motility. However, other reports have shown that a change in the study protocol may affect the absorption of co-administered drugs via an effect thought to occur by a decreased rate of gastric emptying. The present study therefore seeks to evaluate the effect of Cimetidine on Paracetamol pharmacokinetics when it is administered one hour prior to Paracetamol administration and compare this to the effect produced when Hyoscine bromide; which is known to delay gastric emptying is administered prior to Paracetamol. Sixteen healthy volunteers participated in the study which was conducted in two phases. In the first phase, 1g of Paracetamol was administered orally to the volunteers and in the second phase, the volunteers were divided into two groups of eight subjects each and the first group was given 400mg of Cimetidine orally 1 hour prior to Paracetamol administration while the second group received 10mg of Hyoscine bromide orally prior to Paracetamol administration. Plasma concentration of Paracetamol was determined using a validated spectrophotometric method. Pharmacokinetic parameters were calculated using standard non-compartmental model equations. The study found that delayed administration of Paracetamol after administration of Cimetidine led to statistically significant changes (p<0.05) in some of the pharmacokinetic parameters especially the absorption parameters such as Ka, $t_{1/2}\alpha$, Cmax and Tmax as compared to the control group receiving Paracetamol alone. These effects mirror the effects produced when the anticholinergic agent Hyoscine bromide was administered prior to Paracetamol admnistration. However, the elimination parameters of Paracetamol were not significantly altered by Cimetidine.

Keywords: Cimetidine, Gastric emptying, Hyoscine bromide, Paracetamol, Pharmacokinetics drug interactions.
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INTRODUCTION

Drug interactions are pharmacodynamic, pharmacokinetic, or clinical responses to the administration of a drug combination that differs from the known effects of the individual drugs administered alone. The clinical consequences of drug interactions may be antagonistic, additive, synergistic, or idiosyncratic, resulting in treatment failure, increased pharmacologic effect, or toxic reactions, which may be serious or fatal. Due to the fact that most drugs are detoxified by the liver, the most important of the pharmacokinetic drug interactions involve drug

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metabolism usually entailing the induction or inhibition of the cytochrome P450 (CYP450) enzyme system which is responsible for oxidative-reductive metabolism. However, it has also been recognized that drug interactions mediated via alterations in drug absorption, distribution and excretion may also be clinically significant.

Cimetidine is an imidazole derivative, H_2-receptor antagonist that is used primarily to decrease gastric acid secretion in the management of Peptic ulcer and other acid hypersecretory disorders [1]. The drug interactions of Cimetidine have been extensively studied and are well recognized in clinical practice due to their significance. Several different mechanisms have been proposed for Cimetidine-related drug interactions. These mechanisms include: Impaired hepatic drug metabolism due to inhibition of hepatic microsomal enzymes; reduced hepatic blood flow resulting in decreased clearance of drugs that are highly extracted by the liver; increased potential for myelosuppression when administered concurrently with other drugs capable of causing myelosuppression; altered bioavailability of acid-labile drugs [2,3,4].

There has been no consensus on the effect of Cimetidine on gastric motility. A survey of existing literature shows that most of the studies involving Cimetidine claimed that the drug has no effect on gastrointestinal tract (GIT) motility and hence its effect on the absorption of other orally co-administered drugs cannot be attributed to a possible effect on gastric motility [5,6,7]. However, Garba et al [8] had previously postulated an additional mechanism for Cimetidine- drug interactions, suggesting that in addition to the above mentioned mechanisms, Cimetidine can also cause direct relaxation of GIT smooth muscle which can in turn reduce the rate of gastric emptying & consequently cause a decrease in absorption rate of co-administered drugs. This effect was found to occur when there was a change in the study protocol where rather than administer the two drugs concurrently, Cimetidine is administered one hour before Paracetamol administration. This led to significant changes in Paracetamol pharmacokinetics which resembled the effect obtained when Paracetamol is given together with antimuscarinic agents.

Hyoscine-N-butyl bromide is a peripherally acting anticholinergic agent used as an abdominal-specific antispasmodic for the treatment of pain and discomfort caused by abdominal cramps, menstrual cramps, or other spasmodic activity in the digestive system. It is a quaternary ammonium compound and a semi-synthetic derivative of scopolamine [9]. Antimuscarinic drugs such as Atropine [10, 11], Propantheline [12, 13] and mepenzolate [13,14], have all been shown to reduce gastric emptying. Gastric emptying is rate limiting in the absorption of many orally administered drugs [15, 16, 17, 18]. It has been shown that negligible absorption of Paracetamol occurs in the stomach, the bulk of an orally administered dose of been absorbed from the small intestine [19]. The rate of Paracetamol absorption therefore depends mainly on the rate of gastric emptying [20] and drugs with antimuscarinic properties such as Propantheline [21], Desmethylimipramine [22] and Atropine [23] which reduce the rate of gastric emptying have been shown to delay Paracetamol absorption.

Paracetamol is an analgesic and antipyretic agent with negligible anti-inflammatory properties. Oral Paracetamol is rapidly and almost completely absorbed from the gastrointestinal tract primarily in the small intestine, with negligible absorption occurring in the stomach [24, 25, 26]. The relative bioavailability ranges from 85% to 98% [27]. Peak levels of 10-20 mg/l are achieved within one to 2 hours after the administration of a 1000-mg dose orally. Serum concentrations between 10 and 20 μg mL^{-1} are generally considered to be therapeutically effective, while>150 μg mL^{-1} may produce hepatic necrosis [28]. Absorption is influenced by the presence of food [29], and also by the rate of gastric emptying [21]. Paracetamol does not bind strongly to tissues [30, 31]. It appears to be widely distributed throughout most body fluids except fat. The apparent volume of distribution of Paracetamol is approximately 0.7 to 1.0 L/kg in children and adults [32]. A relatively small proportion (10% to 25%) of Paracetamol is bound to plasma proteins [33]. Paracetamol is primarily metabolized in the liver and involves 3 main pathways: conjugation with glucuronide (about 55% of a therapeutic dose); conjugation with sulphate (about 30%); and oxidation via the cytochrome P450 (CYP450) enzyme pathway. The oxidative pathway forms a reactive intermediate, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is detoxified by conjugation with glutathione to form inert cysteine and mercapturic acid metabolites [34]. The principal CYP450 isoenzyme involved in vivo appears to be CYP2E1. Two additional minor pathways are involved in Paracetamol metabolism: hydroxylation to form 3-hydroxy- Paracetamol and methoxylation to form 3-methoxy- Paracetamol [35]. These catechol metabolites are further conjugated with glucuronide or sulphate. The glucuronide-, sulphate-, and glutathione-derived metabolites lack biologic activity [36]. The elimination half life of Paracetamol in healthy adults is approximately 2 to 3 hours in the usual dosage range [37, 38]. It is about 1.5 to 3 hours in children, and about 1 hour longer in neonates [31] in cirrhotic patients [39] and in some ethnic groups (i.e., Nigerians, Hong Kong Chinese) [33, 40, 41]. Approximately

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3.5% of Paracetamol is excreted unchanged in the urine [42]. Slight differences have been seen in ethnically distinct populations (e.g., Asian, Spanish) [43, 44, 45, 46].

The present study therefore seeks to investigate the oral pharmacokinetics of Paracetamol after its delayed administration sequel to Cimetidine administration, to observe if there will be any changes in Paracetamol kinetics and to compare the observed changes to the variations produced by the administration of Paracetamol with an anticholinergic agent represented by Hyoscine bromide. Interpretation of the information obtained from the study will aid in making an informed decision on the veracity of the earlier suggested hypothesis that Cimetidine may have an "anticholinergic-like" effect on other co-administered drugs if there is a substantial time interval between the administrations of the two drugs.

MATERIALS AND METHODS

Materials
Paracetamol Reference Tablets (German Pharma Health Fund (GPHF) minilab reference); Manufacturing Date: 03/2009, Expiry Date: 02/2012, Lot No: MFE262/05D05 was obtained from the Pharmaceutical Chemistry Department, University of Jos. Paracetamol 500mg Tablets (Emzor®; Manufacturing Date: 07/2009, Expiry Date: 07/2012, Batch No: 4905J), Cimetidine 400mg tablets (RGI International; Manufacturing Date: 08/2010, Expiry Date: 07/2013, Batch No: RGI001), and Hyoscine N-butyl bromide 10mg tablets (Manufacturing Date: 05/2010, Expiry Date: 05/2015, Batch No: H083) were obtained from a Pharmacy retail outlet in Jos, Nigeria. Clifton Centrifuge (Nickel Weston-S-mare Electro Ltd Avon) was used for centrifugation and UV-Visible Double beam Spectrophotometer (Shimadzu model 1250, Japan) was used for the assay. Kymograph + Stimulator (Bioscience model 10550) was used for the invitro motility test. All chemicals used were of analytical grade and obtained from the Department of Pharmaceutical Chemistry, University of Jos. These include: Trichloroacetic acid (Riedel de Haen, Germany), Sulphamic acid (Riedel de Haen, Germany), Sodium Hydroxide (BDH chemical LTD, Poole England), Sodium Nitrite (BDH chemical LTD, Poole England), Conc. Hydrochloric acid (Sp. Gr. 1.18, BDH chemical LTD, Poole England).

Subject treatment, sampling time and collection
A total of sixteen (16) healthy, non-smoking human volunteers, both male and female weighing between 55 and 75 kg and between the ages of 18 – 28 years, participated in the study with their full consent. Approval was sought and obtained from the Ethics committee of the Jos University Teaching Hospital (JUTH) after they reviewed the study protocol. The nature, aims, and objectives of the study was explained to the subjects. All the subjects gave their written informed consent to participate.

Subjects were randomly selected and were certified to be medically fit, non-alcoholic, non-smokers and were instructed to abstain from taking any drugs one month prior to the study. After obtaining informed consent from them, the participants were then divided into two (2) groups with eight (8) individuals per group. The study was conducted in two phases:

In the first phase (Phase I), after an overnight fast, 5 ml of venous blood was taken pre-dose. Each member of both groups was then given Paracetamol (2 x 500 mg tablets) orally alone with 200 ml of water. A 5 ml of venous blood was then collected at 0.00, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 and 6.00 hours respectively. A wash out period of Two (2) weeks was then allowed for all the volunteers in both groups before commencement of the second phase.

In the second phase (Phase II), after an overnight fast, 5 ml of venous blood was taken pre-dose. Hyoscine-N-Butyl Bromide (10 mg tablet) (Group I) and Cimetidine (400 mg tablet) (Group II) was then administered orally to the volunteers in the two separate groups respectively followed one hour later by Paracetamol (2 x 500 mg tablets). The same procedure as above was then employed in collecting the blood samples.

Sample Pretreatment
All the blood samples taken in both phases were collected into Disodium Edetate treated plastic containers, immediately centrifuged at 3000 rpm for five (5) minutes and the plasma harvested into clean plastic containers, stoppered and stored at -20 °C until analysis.

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Preparation of Paracetamol Stock solution
100 mg of Paracetamol reference powder was weighed and dissolved in about 50 ml of warm distilled water with gentle stirring. The solution was then transferred into a 100 ml volumetric flask and the volume made up to mark with distilled water. This gave a solution of strength 1mg/ml or 1000 mcg/ ml. From this stock solution, several working dilutions of the standard paracetamol solution were prepared to give solutions of strength 50, 100, 200, 300 and 500 mcg/ml.

Construction of calibration Curve and its validation
Five (5) test tubes were taken and labeled appropriately and 0.9 ml of blank plasma was pippetted into each of the test tubes. 0.1 ml each of the working dilutions of standard paracetamol solution prepared above was then used to spike the blank plasma in the test tubes to give final concentration of paracetamol in plasma corresponding to 5, 10, 20, 30 and 50mcg/ml. Each concentration above was prepared in quadruplicate.

Analytical Procedure:
The concentration of Paracetamol in the plasma samples prepared above was then determined using an adaptation of the UV spectrophotometric method of Glynn and Kendal [47]. A 2ml of 15% trichloroacetic acid was added to the 1ml of plasma prepared above and vortex mixed. The sample was then centrifuged at 3000 revolutions per minute for 3 minutes. The clear supernatant obtained was then decanted into another test tube containing 1ml of 6M HCl. Nitrous acid was then generated by addition of 1ml of sodium nitrite solution to the resultant solution. The content of the test tube was then allowed to stand for 2 minutes and 2 ml of Sulphamic acid was then carefully added to neutralize the excess Nitrous acid. Finally, 5ml of 15% Sodium hydroxide was then added. The absorbance of the final solution was then taken at 430nm with the aid of a Shimadzu double beam spectrophotometer against a blank of drug free plasma treated exactly as above.

The plot of absorption against concentration (Beer-Lamberts plot) was obtained from the mean of four replicate determinations with the aid of Microsoft excel (version 2007). The regression equation and the correlation coefficient for the plot were also obtained.

Validation of the Analytical Method
Accuracy of the method was determined by carrying out recovery experiments. Selected concentrations of the paracetamol reference solution were prepared and these were used to spike blank plasma. The samples were then analyzed as in the calibration curve and the absorbencies obtained. The corresponding concentrations were then obtained with the aid of the calibration plot and the percentage recoveries calculated. Precision of the method was assessed by determining inter and intra-day variation, replicate analysis (n = 5) of calibration standards were conducted at three different concentration levels, five times per day on five consecutive days. The stability of the product formed from the reaction was also evaluated. Molar absorptivity, Sandells' sensitivity, regression equations and standard deviations were similarly determined. The limits of detection (LOD) and quantitation (LOQ) were also determined.

Sample Assay
The plasma sample collected from the 2 different phases of the study were then analyzed using exactly the same procedure as described above for the calibration curve and the absorbance values obtained were converted into the corresponding concentrations with the aid of the regression equation obtained from the calibration curve.

Data Handling and Analysis
Pharmacokinetic Analysis
The concentration values obtained for the different sample were then used to plot plasma concentration-time curves for the different individuals on a semi-logarithm graph sheet. Different pharmacokinetic parameters were then determined for individual subjects in both phases using the non-compartmental model. The maximum plasma drug concentration (Cmax) attained and the corresponding time (Tmax) to achieve this were measured directly from the paracetamol plasma-concentration time plot. The area under the curve (AUC) from time 0 to 6 hours was determined using the Linear trapezoidal rule with extrapolation to time infinity ($AUC_{0-\infty}$) by Ct/Kel. The elimination rate constant (Kel) was determined from the slope of the terminal elimination part of the plasma concentration time plot and elimination half life ($t_{1/2}\beta$) was obtained from the equation $t_{1/2}\beta = 0.693/Kel$. The absorption rate constant (Ka) was determined using the residual method while $t_{1/2}\alpha$ was obtained from the equation $t_{1/2}\alpha = 0.693/Ka$. The

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Clearance was calculated as FDose/AUC$_{0-\infty}$ while Volume of distribution (Vd) was obtained from Cl/Kel. The pharmacokinetic parameters are presented as mean ± standard deviation.

Statistical Analysis
Statistical analysis of the data obtained was carried out using the students t-test to establish if there were any significant differences in paracetamol absorption kinetics when it is given alone as compared to delayed administration (one hour) after administration of Hyoscine-N-Butyl Bromide and Cimetidine. This was done using Graph pad Prism 5 for Windows statistical software ($P<0.05$ was considered statistically significant). Results are reported as Mean ± Standard Deviation unless otherwise stated.

Prior to the study, quality control tests (i.e. identification tests, disintegration and dissolution rate tests and assay for content of active ingredient) were carried out on the Cimetidine, Paracetamol and Hyoscine-N-Butyl bromide tablets to be used in the study, using their official Pharmacopeia methods [48] in order to authenticate the tablets.

Invitro Tests on Isolated Rabbit Jejunum
A mature adult rabbit was sacrificed humanely. Its abdomen was then dissected and a portion of the jejunum was obtained. This was then mounted in 0.9% Sodium Chloride (Physiological Saline) solution in an organ bath of a kymograph. The speed of the instrument was then set at 40 mm/sec. Normal contractions of the tissue were then recorded for some seconds to serve as a baseline. A 0.5ml volume of a 0.1mg/ml aqueous solution of Cimetidine was then infused into the organ bath and its effect on the tissue motility was then recorded. The bath was then emptied of its contents and fresh saline was used to rinse the tissue severally to wash out the entire drug.

Several dilutions of the drugs under investigation were then prepared and the effects of these solutions on the motility of isolated rabbit jejunum were also evaluated with the aid of the kymograph in the order shown below:
i.   Cimetidine 2mg/ml and 0.02mg/ml
ii.   Hyoscine-N-Butyl bromide 0.1mg/ml, 0.2mg/ml and 0.02mg/ml
iii.   Acetylcholine 0.002mcg/ml
iv.   Acetylcholine + Cimetidine
v.   Acetylcholine + Hyoscine-N-butyl bromide

Table 1: Extraction Recovery

S/No.	Spiked Conc. (µg/ml)	Conc. Obtained (µg/ml)	% Recovery	% RSD
1.	5	4.77 ± 0.23	95.4	4.82
2.	10	9.26 ± 0.44	92.6	4.75
3.	20	19.41 ± 0.57	97.0	2.94
4.	30	29.32 ± 0.69	97.73	2.35
5.	50	49.45 ± 1.78	98.9	3.59
	Average		96.34	3.69

Table 2: Quantitative parameters for the analytical method.

S/n	Parameter		Observation
1.	Linearity range (mcg/ml)		10-50
2.	Recovery (%)		96.34%
	Precision		
3.	i.	Inter-day variation	1.13 – 1.43 %
	ii.	Intra-day variation	0.85 – 1.37 %
4.	Limit of detection (mcg/ml)		0.26
5.	Limit of quantitation (mcg/ml)		0.86
6.	Molar absorbtivity (Lmol^{-1}cm^{-1})		2.273×10^{4}
7.	Sandells sensitivity (mcg/cm^{2})		0.115×10^{-4}
8.	Correlation coefficient (r^{2})		0.996
9.	Stability (hours)		6

RESULTS

Data for the recovery experiments conducted are presented in Tables 1. The results of the method validation and optimization studies are presented in Table 2. The pharmacokinetic profile of paracetamol when administered alone and when administered an hour after Hyoscine bromide and Cimetidine respectively are presented in Table 3 while Table 4 shows the pharmacokinetic parameters for the three different groups studied. Figure 1 shows the calibration

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curve for Paracetamol in plasma while Figure 2 shows the mean plasma concentration time profiles of paracetamol tablet after a single oral dose (1gram) and when administered an hour after hyoscine bromide and cimetidine.

Table 3: Six hour mean plasma concentration sampling

Time (hours)	Mean plasma concentration SD (mcg/ml)		
	Paracetamol alone	Paracetamol + Hyoscine Bromide	Paracetamol + Cimetidine
0	0	0	0
0.25	6.40 ± 0.26	0	2.79 ± 0.14
0.50	13.5 ± 0.35	3.06 ± 0.21	5.89 ± 0.11
1.00	19.5 ± 0.25	6.89 ± 0.15	8.67 ± 0.09
2.00	11.64 ± 0.22	10.15 ± 1.00	10.9 ± 0.12
3.00	5.57 ± 0.37	6.61 ± 0.08	6.25 ± 0.10
4.00	4.32 ± 0.29	5.73 ± 0.46	5.08 ± 0.41
6.00	2.60 ± 0.24	3.86 ± 0.09	3.1 ± 0.09

Table 4: Pharmacokinetic Parameters of Paracetamol after oral administration (1gram) when administered alone and upon delayed administration (one hour) after administration of Hyoscine Bromide and Cimetidine

S/N	Pharmacokinetic Parameter	Paracetamol Alone	Paracetamol + Hyoscine Bromide	Paracetamol + Cimetidine
1.	Lag time (hr)	0.22 ± 0.009	$0.49 \pm 0.018^*$	$0.29 \pm 0.011^*$
2.	Ka (hr^{-1})	11.84 ± 2.86	$3.55 \pm 0.193^*$	$4.22 \pm 0.44^*$
3.	$t_{1/2}\,\alpha$ (hr)	0.0693 ± 0.019	$0.196 \pm 0.011^*$	$0.17 \pm 0.019^*$
4.	Tmax (hr)	1	2	2
5.	Cmax (mcg/ml)	20.12 ± 2.41	$10.15 \pm 1.00^*$	$10.90 \pm 0.12^*$
6.	AUC$_{0-6}$ (mcg.hr/ml)	48.09 ± 2.76	$35.50 \pm 0.99^*$	$36.55 \pm 1.68^*$
7.	AUC$_{0-inf}$ (mcg.hr/ml)	52.63 ± 3.13	$44.82 \pm 1.06^*$	$42.37 \pm 1.98^*$
8.	K$_{el}$ (hr^{-1})	0.595 ± 0.094	$0.417 \pm 0.024^*$	0.534 ± 0.024
9.	$t_{1/2}\,\beta$ (hr)	1.19 ± 0.20	$1.67 \pm 0.098^*$	1.29 ± 0.062
10.	Cl (ml/hr)	17.18 ± 1.04	20.09 ± 0.48	21.29 ± 1.05
11.	Vd (ml)	0.48 ± 0.096	$0.81 \pm 0.106^*$	$0.62 \pm 0.072^*$

Mean $\pm$ SD (n = 16 for group I and n = 8 for group II and III; P*<0.05 compared to control; AUC, Area under the plasma concentration time curve; Cmax, peak concentration; Tmax, time to attain maximum concentration; $t_{1/2}\beta$, elimination half life; Kel, elimination rate constant; Cl, clearance; Vd, volume of distribution; Ka, absorption rate constant; $t_{1/2}\alpha$, absorption half life
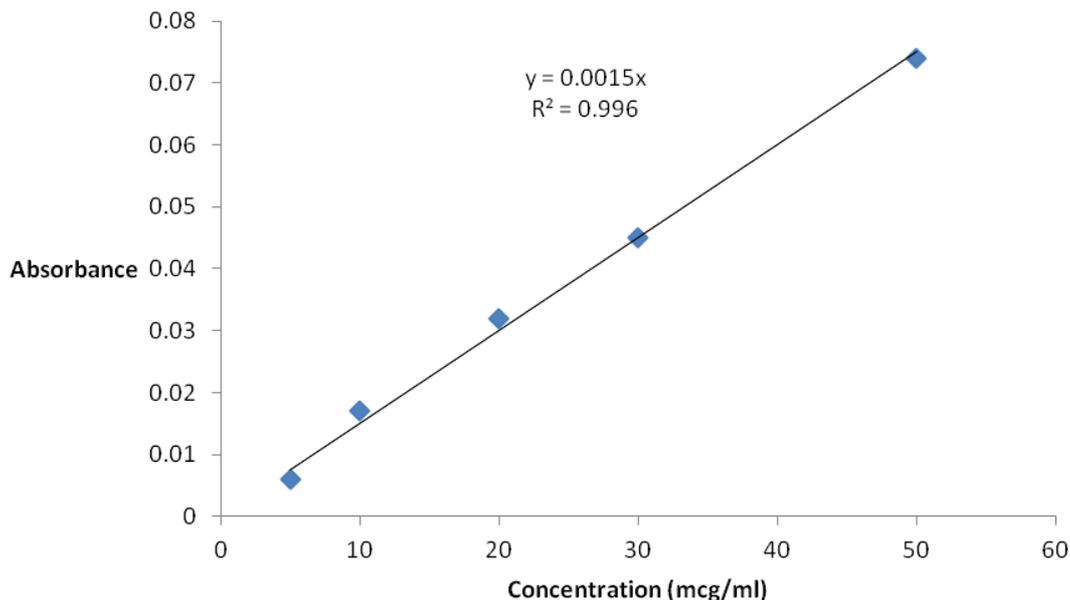
Figure 1: Calibration curve for paracetamol in plasma

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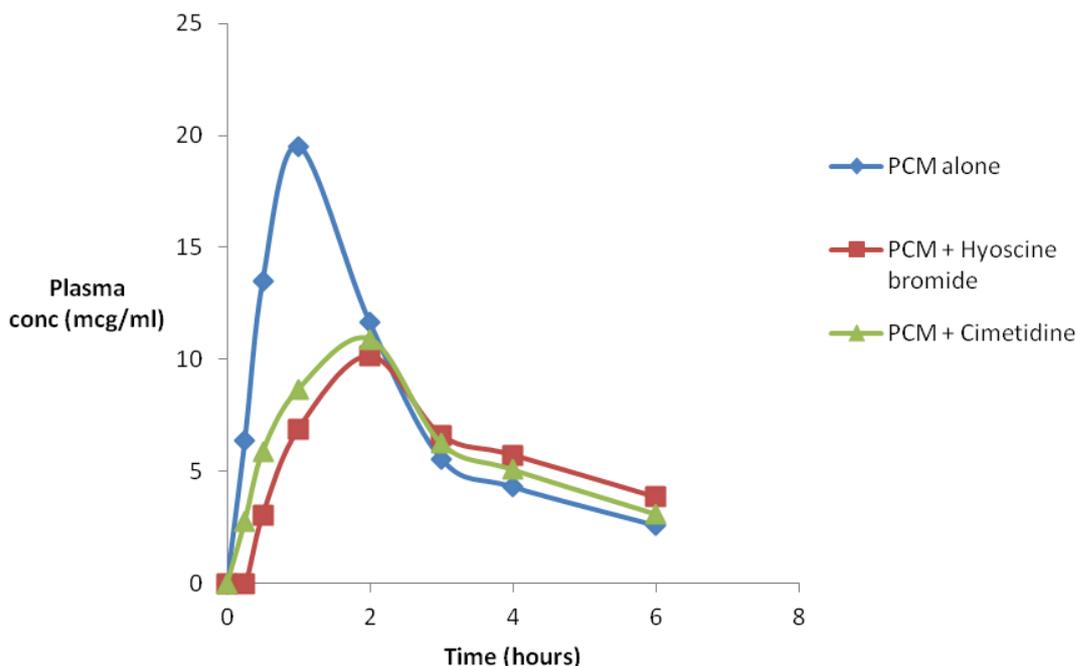
Figure 2: Plasma concentration-time profile of oral Paracetamol (1000 mg) in human volunteers when administered alone and upon delayed administration (one hour) after administration of Hyoscine Bromide and Cimetidine.

DISCUSSION

The calibration curve was found to be linear over the concentration range of 10-50mcg/ml with a correlation coefficient (r^2) of 0.996. Mean percentage recovery of Paracetamol from plasma was found to be 96.34% with % RSD of 3.69%. Considering these and the other validation parameters displayed on table 2, it can be reasonably concluded that the adapted spectrophotometric method is therefore suitable and adequate for the comparative pharmacokinetics and drug interaction study conducted.

The Lag time for the group on Paracetamol alone was found to be 0.22 ± 0.009 hours and this value is in close agreement with previously reported values [49], who reported a lag time of 0.23 hours. On the other hand, the Lag time for paracetamol for the groups taking Hyoscine bromide and Cimetidine increased significantly with values of 0.49 ± 0.018 and 0.29 ± 0.011 hours respectively. These increments are statistically significant ($P<0.05$). The lag time is the time interval between when the drug is administered and when it first appears in blood and for an orally administered drug, it is indicative of how fast the drug is absorbed from the GIT. Since Hyoscine bromide is an anticholinergic agent, this delay in lag time is expected as the drug is able to delay gastric emptying via its blockade of muscarinic receptors in the GIT smooth muscles thereby slowing the movement of Paracetamol from the stomach to the small intestine which is the principal site of its absorption. Also, the lag time increased for the group on Cimetidine and this is a confirmation of the proposed hypothesis that delayed administration of Paracetamol one hour after Cimetidine administration will lead to changes in the absorption kinetics of paracetamol. It can thus be deduced that Cimetidine may have some "anti-cholinergic like" effect on the GIT smooth muscle. It is pertinent to note here that when Cimetidine is administered concurrently with Paracetamol this effect is not observed [7] but is only observed when there is about one hour interval between when the two drugs are administered. This may be attributed to the fact that the peak plasma concentration of Cimetidine, which is the time when its pharmacological properties are expected to be fully manifested, occurs at about one hour after it is administered and this coincides with the time when this effect is observed.

The absorption rate constant (Ka) was found to have decreased significantly ($P<0.05$) for the Hyoscine bromide group and the Cimetidine group (3.55 ± 0.193 and 4.22 ± 0.44 hr-1 respectively) as compared to the control group that received paracetamol alone (11.84 ± 2.86 hr-1) showing that the test drugs slowed down the rate of paracetamol

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absorption from the gastrointestinal tract. Also, this pattern is mirrored by the values obtained for the absorption half life ($t_{1/2}\alpha$) which increased significantly for the two test groups as compared to the control also showing that the time taken for the half of the drug initially present to be absorbed was significantly increased by both Hyoscine bromide and Cimetidine. Paracetamol is weakly acidic (pKa 9.5) and it is mainly absorbed from the small intestine probably due to the large surface area presented for absorption. Heading et al [19] had previously shown that paracetamol absorption is directly dependent on the rate of gastric emptying, as this will influence the rate of delivery of the drug to the small intestine which is the principal site of its absorption as previously stated. There are other factors such as the presence of food, volume, composition, tonicity, temperature and pH of the stomach contents [50], that can also influence the rate of gastric emptying but since these other factors were kept relatively constant, it can be deduced that the delay in the absorption of paracetamol upon delayed administration with Cimetidine is directly attributable to the effect of the Cimetidine in slowing down the rate of gastric emptying.

The maximum plasma concentration (Cmax) attained by paracetamol alone (20.12 ± 2.41 mcg/ml) was found to be in agreement with published values in literature which stated a range of 10-20mcg/ml [51]. However, the Cmax for paracetamol was significantly reduced in both the Hyoscine bromide and Cimetidine groups (10.15 ± 1.00 and 10.90 ± 0.12 respectively). This shows that the two drugs were able to significantly decrease the extent of paracetamol absorption. This effect can again be linked to the anticholinergic action of Hyoscine bromide, as GIT motility is principally controlled by the parasympathetic nervous system which serves to promote increased motility and inhibition of its activity as can be mediated by anticholinergics will lead to a slowing of GIT motility with the attendant effects on the movement of the other drugs form the stomach to the small intestine where a larger percentage of drug absorption takes place. Cimetidine was also found to produce a similar action and this can once again be attributed to its postulated effect in slowing down gastric emptying. This finding is also in close agreement with the previously published study by Garba et al [52] who reported an almost 50% decrease in the Cmax of Paracetamol from 30.8 ± 1.4 mcg/ml to 16.02 ± 0.7 mcg/ml, when Cimetidine was administered one hour prior to paracetamol administration.

Another pointer to validate this action of Cimetidine in delaying gastric emptying is the fact that cimetidine is a known inhibitor of CYP2E1 isoenzyme while Paracetamol is a substrate for the same isoform, hence we will expect an increase in plasma levels of paracetamol upon its administration with Cimetidine if the interaction occurring in this group was due to enzyme inhibition but the opposite effect was observed with a large decrease in plasma paracetamol concentration upon delayed administration with cimetidine showing that the cimetidine actually reduces paracetamol absorption probably via delayed gastric emptying.

Furthermore, Tmax which indicates the rate of absorption of the paracetamol was increased significantly from one hour for the control group to about two hours for both the Hyoscine bromide and Cimetidine group showing that both drugs were able to decrease both the rate and extent of paracetamol absorption in a statistically significant manner ($P<0.05$). All the values of tmax above however still fall within the previously reported range of $1 - 2$ hours [51].

The value of area under the curve from time zero to infinity ($AUC_{0-\infty}$) which is indicative of the overall extent of paracetamol absorption/ bioavailability decreased significantly ($P<0.05$) in both the groups that received Hyoscine bromide (44.82 ± 1.06) and Cimetidine (42.37 ± 1.98) prior to paracetamol as compared to the control group which received only paracetamol (52.63 ± 3.13). This was expected for the Hyoscine bromide due to its anticholinergic effect which will delay gastric emptying and slow down the absorption of the paracetamol which takes place predominantly in the small intestine. A similar effect was observed with Cimetidine upon the delayed administration of Paracetamol and this is in tandem with the earlier mentioned changes in the absorption parameters. The area under the curve from time zero to six hours (AUC_{0-6}) also showed a similar pattern as above.

The elimination half life ($t_{1/2}\beta$) was found to be 1.19 ± 0.20 for the group on Paracetamol alone as against 1.67 ± 0.098 and 1.29 ± 0.062 for the groups also receiving Hyoscine bromide and Cimetidine respectively. This parameter showed a statistically significant difference ($P<0.05$) when comparing the Paracetamol group with the Hyoscine bromide group but comparing the paracetamol group with the Cimetidine group, there was no significant change in the elimination half life of Paracetamol. However all the values obtained above are still within the previously reported ranges for this parameter in normal individuals which is between 1-4 hours [51,53]. The elimination rate constants (Kel) of paracetamol for the three different groups also fall within documented values.

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The Clearance (Cl) value for the Paracetamol alone group was obtained as 17.18 ± 1.04 ml/hr while that for the Hyoscine bromide group and the Cimetidine group were 20.09 ± 0.48 and 21.29 ± 1.05, these values all fall within the range reported by Perucca and Richens [54]. Clearance is a parameter that represents the drug elimination from the body and it encompasses both renal clearance of the drug and hepatic clearance (metabolism).

From the foregoing, it can therefore be inferred that both Hyoscine bromide and Cimetidine in the study did not produce any changes in the elimination kinetics of paracetamol. This is not surprising since the study is a single dose study, it is not expected that any changes in the metabolism and excretion of paracetamol would have been produced by the test drugs.

The volume of distribution (Vd) which is a measure of the drugs distribution was significantly increased from a value of 0.48 ± 0.096 for the group on paracetamol alone to 0.81 ± 0.106 and 0.62 ± 0.072 for the groups on Hyoscine bromide and Cimetidine respectively. These changes were statistically significant at $P<0.05$ but fall within the range of $0.69 – 1.36$ L/Kg as reported by Vozeh et al and Forrest et al, [53, 55] except for that for the group on paracetamol alone which was slightly below this range. This can be explained on the basis of the wide variability in the elimination of Paracetamol in certain ethnic groupings of which Nigerians have been found to be inclusive [40]. The pharmacokinetic sampling times were from pre-dose to six hours post dose. It is believed that this was sufficient to give a true reflection of the passage of the drug through the body since the half life of paracetamol in healthy human volunteers is about 2 hours and the minimum sampling duration acceptable for pharmacokinetic studies is three (3) times the half life of the drug and this was adapted in the study.

The results of the in vitro tests carried out on isolated rabbit jejunum also showed that the varying concentrations of Hyoscine bromide tested on the tissue were able to decrease the motility of the tissue as seen by the decreased frequency and amplitude of contractions. The drug was also able to inhibit Acetylcholine induced increases in tissue contraction. These effects are expected as the Hyoscine bromide by virtue of its anticholinergic effect would block the muscarinic receptors on the tissue and prevent Acetylcholine from stimulating them and since GIT motility is principally under the control of the parasympathetic system, interference with its functioning will certainly lead to the observed effect. On the other hand, the various concentrations of Cimetidine tested were able to decrease the motility of the isolated tissue but not to the same extent as was observed with Hyoscine bromide. Cimetidine was however not able to significantly inhibit Acetylcholine induced increases in motility of the tissue as compared to the inhibition of Ach induced increases in tissue motility as seen with Hyoscine bromide. It can thus be deduced that though Cimetidine may have some effect in slowing down the motility of intestinal smooth muscles, this effect may not necessarily be mediated via interference with the cholinergic system but via some other mechanism.

CONCLUSION

It can therefore be concluded that delayed administration of Paracetamol one hour after Cimetidine administration is capable of significantly reducing the rate and extent of paracetamol absorption in a manner similar to the effect produced by anticholinergics which are known to reduce the rate and extent of paracetamol absorption via reductions in gastric emptying time. However, since the cimetidine was unable to effectively prevent Acetylcholine induced increases in motility of the isolated rabbit jejunum, it cannot be conclusively established that its action in slowing GI motility and gastric emptying is mediated through a direct action on Muscarinic receptors.

REFERENCES

[1] D.G. Seibert and L.M. Gangarosa, In: R.C. Craig and R.E. Stitzel (Ed.), Modern Pharmacology with Clinical Applications, 5th Edition (Lippincott, Williams and Wilkins, **2003**), p.479.
[2] T.L. Schwinghammer, *Am. J. Hosp. Pharm.* **1981**, 38 (12): **1976**-**1978**
[3] E.M. Sorkin and D.L. Darvey, *Drug Intel. Clin. Pharm.*, **1985**, 17 (2): pp. 110-120.
[4] J.R. Powell and K.H. Donn *Am. J. Med.,* **1984**, 77 (suppl. 5B): 60.
[5] C.T. Richardson, J.H. Walsh and M.I. Hicks, *Gastroenterology*, **1976**, 71(1): 19-23.
[6] J.A. Forrest, P. Adriaenssens, N.D. Finlayson and L.F. Prescott, *Eur. J. Clin. Pharmacol.*, **1979**, 15(6):427-431.
[7] M.M. Chen and C.S. Lee, *J. Clin, Pharmacol*, **1985**, 25(3): 227-229
[8] M. Garba, M.T. Odunola and B.H. Ahmed. *Eur. J. Drug Metab Pharmacokin., 1999,* 42: 159-162 (1999).
[9] B.G. Katzung, Basic & clinical pharmacology, McGraw-Hill Medical, **2003**, 9th Edition.
[10] J.M. Hilton and G.P. Lewis *J. Physiol.*, **1955**, 128: 235-248.

_____

[11] A.W. Kay and A.N.Smith AN, *Br. J. Pharmacol.*, **1956**, 11: 231-235.

[12] I.R. Schwartz, E. Lehman, R. Ostrove and J.M. Seibel, *Gastroenterology*, **1953**, 25: 416-430.

[13] S.H. Kraines *Ill. Med. J.*, **1957**, 110: 157-160.

[14] M.S. Kleckner, *Clin. Res. Proc.*, **1957**, 5: 19-20.

[15] R.R. Levine, *Am. J. Dig. Dis.,* **1970**, 15, 171-188.

[16] L.F. Prescott, *Med. Clin. N. Am.*, **1974** 58: 907-916.

[17] W.S. Nimmo, *Clin. Pharmacokinet.*, **1976**, 1: 189-203.

[18] M. Gibaldi. In: Eds L.F. Prescott and W.S. Nimmo (Eds.), Drug absorption, (ADIS Press, Auckland, **1979**) pp. 1-5.

[19] J.A. Clements, R.C. Heading, W.S. Nimmo and L.F. Prescott, *Clin. Pharmacol. Ther.*, **1978**, 24(4):420-31.

[20] R.C. Heading, J. Nimmo, L.F. Prescott and P. Tothill, *Br. J. Pharmacol.* **1973**, 47:414-421.

[21] W.S. Nimmo, R.C. Heading, R. Tothill and L.F. Prescott, *Br. Med. J.*, **1973**, 1: 587.

[22] R.C. Hall, *Postgrad. Med. J.* **1976**, 52: 139-142.

[23] M.U. Rashid and D.N. Bateman, *Br. J. Clin. Pharmacol.*, **1990**, 30: 25-34.

[24] J.R. Gwilt, A. Robertson, L. Goldman and A.W. Blanchard, *J. Pharm. Pharmacol.* **1963**, 15: 445.

[25] T. Grattan, R. Hickman, A. Darby-Dowman, M. Hayward, M. Boyce and S. Warrington, *Eur. J. Pharm. Biopharm.,* **2000**, 49(3):225-229.

[26] M.J. Toes, A.L. Jones and L. Prescott, *Am. J. Ther.*, **2005**, 12(1):56-66.

I.   McGilveray, G. Mattok, J. Fooks, N. Jordan and D. Cook, *Can J. Pharm. Sci.*, **1971**, (6):38-42.

[27] C.L. Winek, *Clin. Chem.*, **1976**, 22: 832-835.

[28] Mattok GL and L.J. McGillivray. *Revue Canadienne de Biologie*, **1973**, Montreal, 32 (Suppl.), 77.

[29] D.N. Bailey, *J. Anal. Toxicol.*, **1997**, 21(1):1-4.

[30] L. Prescott (**2001**) Paracetamol (Acetaminophen): A Critical Bibliographic Review. 2nd Ed. New York, NY: Taylor & Francis Inc.

[31] P. Birmingham, M. Tobin, T. Henthorn, S. Kennedy-Mooney, F. Smith and C. Cote, *Anesthesiology.*, **1996**, 85(3A):A1105.

[32] G. Levy, *Arch. Intern. Med.*, **1981**, 141:279-281.

[33] J.R. Mitchell, S.S. Thorgeirsson, W.Z. Potter, D.J. Jollow and H. Keiser, *Clin. Pharmacol. Ther.*, **1974**, 16(4):676-684

[34] J.T. Slattery, T.I. McRorie, R. Reynolds, T.F. Kalhorn, E.D. Kharasch, and A.C. Eddy, *Clin. Pharmacol. Ther.*, **1989**, 46(5):591-597.

[35] J. Koch-Weser, *N Engl J Med.*, **1976**, 295(23):1297-1300.

[36] B. Bannwarth, P. Netter, F. Lapicque, P. Gillet**,** P. Pere, E. Boccard, *Br. J. Clin. Pharm.,* **1992**, 34(1):79-81.

[37] M.D. Rawlins, D.B. Henderson and A.R. Hijab, *Eur. J. Clin. Pharmacol.*, **1977**, 11(4):283-286.

[38] J.P. Villeneuve, G. Raymond, J. Bruneau, L. Colpron and G. Pomier-Layrargues, *Gastroenterologie Clinique et Biologique.*, **1983**, 7(11):898-902.

[39] O.Q. Yin, B. Tomlinson, A.H. Chow, and M.S. Chow, *Int. J. Pharm.*, **2001**, 222(2):305-308.

[40] C.P. Babalola, F.A. Oladimeji and M.N. Femi-Oyewo, *West Afr. J. Med.*, **2004**, 23(1):10-14.

[41] J.O. Miners, N.J. Osborne, A.L. Tonkin and D.J. Birkett, *Br. J. Clin. Pharmacol.*, **1992**, 34(4):359-362.

[42] J. Critchley, G. Nimmo, C. Gregson, N. Woolhouse and L. Prescott, *Br. J. Clin. Pharmacol.*, **1986**, 22:649-657.

[43] N.J. Osborne, A.L. Tonkin and J.O. Miners, *Br. J. Clin. Pharmacol.,* **1991**, 32(6):765-767.

[44] H. Lee, T. Ti, Y. Koh and L. Prescott, *Eur. J. Clin. Pharmacol.*, **1992**, 43:81-84.

A.   Esteban, R. Calvo and M. Perez-Mateo, *Eur. J. Drug Metab. Pharmacokinet.*, **1996**, 21(3):233-239.

[45] J.P. Glynn and S.E. Kendal *Lancet*, **1975**, 1: 1147 – 1148.

[46] British Pharmacopoeia **2002**, The Stationery office London.

[47] J.A. Kolawole and A. Maduenyi, *Eur. J. Drug Met.Pharmacokinet.*, **2004**, 29 (1): 25-29.

[48] J.N. Hunt In: F. Avery-Jones (Ed.), Modern Trends in gastroenterology (Butterworth, London, **1958**). pp 163-176.

[49] T.J. Meredith and R. Goulding, *Postgrad. Med. J.*, **1980**, 56: 459-473

[50] M. Garba, A. Mustapha, A.K. Haruna and I. Abdu-Aguye, In: 2ⁿᵈ International Symposium for drug interactions, **1997**, Baltimore, USA.

[51] S. Vozeh, O. Schmidlin and W. Taeschner, *Clin. Pharmacokinet.*, **1988**, 15: 254-282.

[52] E. Perucca, and A. Richens, *Br. J. Clin. Pharmacol.,* **1979**, 7: 201-206.

[53] J.A. Forrest, J.A. Clements and L.F. Prescott, *Clin. Pharmacokin.,* **1982**, 7(2): 93-107.