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Effect of Short-Term Concomitant Use of Polyherbal Mixture on the Bioavailability of HIV Protease Inhibitors and Organ Damage in Rats

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ABSTRACT

Many herbal remedies are used as complementary therapy to combination antiretroviral therapy (cART). Contrary to the general assumptions that herbal remedies are harmless because of the natural source, many have been found to be toxic. This study seeks to assess the effect of concomitant administration of HIV protease inhibitors (PIs) Atazanavir/ritonavir (Atz/r) and Lopinavir /ritonavir (Lpv/r) with marketed poly-herbal mixture "Goko cleanser herbal mixture (GCHM)". A modified *in vitro* study of the release profile of Atz/r and Lpv/r was evaluated using dissolution apparatus II. A 15-day sub-acute toxicity test was carried out with GCHM administered orally at 0.5 mL/kg and 1.5 mL/kg simulating low and high doses, respectively together with either Atz/r (10 mg/kg b.wt) or Lpv/r (5 mg/kg b.wt) to 4-week old Wistar rats. Histopathology of the heart and liver, haematological and biochemical analyses of blood obtained via cardiac puncture was carried out. The presence of the poly herbal formulation reduced the release of both PIs *in vitro* irrespective of the media used. There was a significant decrease in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ($P < 0.05$) at a dose of 1.5 mL/kg GCHM compared to the control. Photomicrographs of the liver showed evidence of enlarged nuclei and degenerated hepatocytes in the high dose group treated with Atz/r / GCHM. Concomitant administration of the poly-herbal formulation GCHM, alongside PIs adversely affected pharmaceutical availability which may influence the bioavailability of the PIs.

Keywords: *Protease inhibitors, Pharmaceutical availability, Polyherbal formulations, Antiretrovirals.*

Introduction

The World Health Organization defines herbal remedies as herbs, herbal materials, herbal preparations and finished herbal products, used to treat a multitude of ailments throughout the world.[1, 2] Depending on their chemical constituents one herbal remedy differs from the others in its therapeutic effects and toxicity.[3]

Many of these herbal remedies are used as complementary therapy to combination antiretroviral therapy (cART).[3, 4] The assumptions that herbal remedies are harmless because they are directly obtained from a natural source has been disputed as many have been found to be toxic.[6] Safe herbal remedies are being identified and its use is encouraged while the use of harmful herbal products is discouraged.[6,7] Unfortunately, many consumers do not know which herbal remedies are safe thus, general acceptance or rejection of the herbal products.[7] It was estimated that over 70% of HIV patients taking herbal remedies denied taking them when asked by medical practitioners [8]. HIV/AIDS is a chronic illness that confines the patient to a lifelong treatment regimen of cART. Patients generally tend to utilize cART with complementary and traditional medicines (CATM) to achieve better response.[9] The effects of this concomitant administration of cART and CATM hasn't been well studied.

Studies have shown that there is an interaction between antiretroviral drugs and herbs. A significant decrease in the steady-state and area under the curve of indinavir and saquinavir, has been demonstrated when co-administered with complementary medicines (CAMs), St. John's wort with indinavir; vitamin C with indinavir[10] and garlic with saquinavir. [11,12] A two-week regimen of Phyto Nova Sutherlandia SU1 tablets which contain *Sutherlandia frutescens* plant material significantly reduced the steady-state and AUC of a single dose of Atz/r in healthy male subjects, implying that the bioavailability of Atz/r may be reduced in the presence of *Sutherlandia frutescens* [13]. Dr Iguedo's Goko® cleanser herbal mixture (GCHM) is one of the most widely used bitters in Nigeria. It is a polyherbal formulation containing *Vernonia amygdalina* , *Saccharum officinarum*, *Allium sativum* , *Cajanus cajan* amd *Zingiber officinale*. These combination of herbs are rich in volatile oils, saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinone , edotides , and sesquiterpenes.[14,15] This study seeks to assess the effect of the polyherbal mixture GCHM on the *in vitro* dissolution of Atazanavir/ritonavir and Lopinavir /ritonavir and on organ damage *in vivo* when they are concomitantly administered in Wistar rats.

Materials and Methods

Chemicals

Atazanavir (Atz/r) and Lopinavir (Lpv/r) analytical standard samples were obtained from Sigma Aldrich St. Louis, MO. Drug samples of Atazanavir ® containing Atz/r (Lot number 3039513 Manufacturing date 04/2015 Expiry date 03/2017, NAFDAC No. A4-1019) and Alluvia® containing Lpv/r (Lot number 1036534 Manufacturing date 01/2015 Expiry date 12/2018, NAFDAC No. A4-1004) were utilized for this study. Potassium dihydrogen phosphate (Thomas Baker® UK), sodium bicarbonate (Sigma Aldrich® USA), Sodium hydroxide (Thomas Baker® UK), Methanol

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(Sigma Aldrich® USA), Hydrochloric Acid (Sigma Aldrich® USA) and LC grade water (Omnisolv®,) was purchased from EMD Millipore Corp. (Billerica, MA, USA). Water was purified by reverse osmosis and filtration through a Milli-Q purification system (Millipore, Milford, and M.A, USA). All solvents and reagents used were of analytical grade and the dissolution media used were always freshly prepared. DR IGUEDO GOKO® cleanser herbal mixture (GCHM) was purchased from the patent and proprietary medicine vendors in Lagos, Nigeria (Batch number 005, National Agency for Food Drug Administration and Control NAFDAC No. AL-0804L, manufacturing date 03/2015, expiry date 02/2017). This research was conducted in 2016 and all the polyherbal formulations and marketed protease inhibitors used were within their shelf lives.

Modified in-vitro dissolution study of Atazanavir 200 mg tablet and Lopinavir 150 mg tablet

Dissolution of each of the PIs was carried out in triplicate, using USP apparatus-II Erweka DT 600 Paddle at 37 ± 0.5°C in 900 mL simulated intestinal fluid pH 6.8 at 50 rpm. At pre-determined time intervals, 5 mL of sample was withdrawn and filtered with the aid of a 0.45 µm millipore filter. Reconstitution for HPLC analysis was carried out and replacement of the withdrawn volume was consistently maintained after each withdrawal cycle as described by Piscitelli *et al.*[11, 12]

Dissolution behaviour of the PIs was studied in the presence of the polyherbal bitters using a modification of the method by Ilomuanya *et al* [16]. In each dissolution vessel containing 890 mL of dissolution media, one 200 mg Atazanavir tablet was introduced together with 10 mL of GCHM, at predetermined time intervals 5ml aliquot samples were withdrawn and replaced with 5 mL of the dissolution medium. The withdrawn samples were filtered thrice using 0.45 µm millipore filter and reconstituted for HPLC analysis as described by Piscitelli *et al.*[11, 12] The dissolution study was carried out using 150 mg Lopinavir tablet at pH 1.2 and 6.5.

HPLC Analysis

Using a validated method by Rezk *et al*,[17] the samples were analyzed. Thirty (30) microliters were injected into HPLC system connected to an ultraviolet spectrophotometer, with an aqueous mobile phase (10 mM ammonium acetate and acetonitrile at 35:65 v/v). The elution was isocratic at a flow rate of 1 mL/min. The HPLC system consisted of an Alliance 2695 Separations module and a 2996 Waters photodiode array UV detector coupled to empower data acquisition software (Waters, Milford, MA, USA). A Luna C18 (2) (5 µm, 150 × 2.0 mm ID) column (Phenomenex, USA) protected by a Luna C18 guard column (Phenomenex, USA) with the same internal diameter was used to achieve chromatographic separation. The mobile phase was filtered under reduced pressure through a 0.45 µm polyvinylidene fluoride (PVDF) membrane (Durapore, Millipore, Bedford, MA, USA) and degassed using an Eyela Aspirator A-25 (Tokyo Rikakikai Co. Ltd., Tokyo, Japan) prior to use. The column was thermostated at 30ºC under these conditions, the runtime was 8 min, Atz/r was then detected at 242 nm and Lpv/r was detected at 210 nm.

Animal study

The Lagos University Teaching Hospital Research Ethics Committee of the College of Medicine, University of Lagos provided ethical approval for the study (CM/HREC/02/16/002). All procedure was in compliance with the American Psychological Association guidelines for ethical conduct in care and use of non-human animal in research.[18]

One hundred and fifty (150) male Wistar rats weighing between 190 to 220 grams were used in the study. The rats were kept in an environmentally controlled breeding room (temperature 25ºC ± 1.2°C, humidity 60% ± 5%, and 12-hour dark/light cycle) with free access to water and standard chow. They were allowed to acclimatize to their new environment for 7 days before the commencement of the study.

Dose selection

LD_{50} data is usually utilized to determine the doses of herbal formulations.[19] GCHM has been deemed safe for human consumption by NAFDAC and the dose that was selected for the animal study as low dose was half the recommended dose by the manufacturers while the high dose was twenty times the normal dose in line with established LD_{50} data for the constituents of GCHM.[20] The dose of the protease inhibitors was given according to body weight [21] and thus extrapolated to fit the weight of the Wistar rats utilized in the study.

Sub-acute toxicity test

A 15-day sub-acute toxicity test was carried out with GCHM administered. The rats were grouped into eight groups of 10 rats per group. All drugs were administered via oral gavage as a single daily dose for 15 days. The dose of protease inhibitors and bitters mimicked the exact dose given in humans.[13,21] The dose of the GCHM used in this study is in consonance with what is written on the instruction label for human use.

Group I: Atz/r 4.28 mg/kg bwt
Group II: Lpv/r 10 mg/kg bwt
Group III: Atz/r 4.28 mg /kg bwt + 0.5 mL/kg of GCHM
Group IV: Atz/r 4.28 mg /kg bwt + 1.5 mL/kg of GCHM
Group V: Lpv/r 10 mg/kg bwt + 0.5 mL/kg of GCHM
Group VI: Lpv/r 10 mg/kg bwt + 1.5 mL/kg of GCHM
Group VII: 1.5 mL/kg of GCHM
Group VIII: distilled water (DW) 1.5 mL/kg.

At the end of the 15-day period, their blood was analyzed for haematological and blood chemistry studies, the animals were humanely sacrificed and their organs examined pathologically.

Clinical observations

Throughout the duration of the study, the rats were observed daily and evaluated for the presence of piloerection, salivation, twitching, difference in food and water intake as well as behavioural changes.

Statistical analysis

The data from this study were analyzed using Microsoft Excel sheet and SPSS Inc. version 11.0 Chicago Illinois. A comparison of the standard with mean values was evaluated via ANOVA in combination with Tukey's post-hoc test was used to identify means of data that were significantly different from each other, at 95% confidence interval ($p \leq 0.05$) with the quantitative variables expressed as mean ± SD.

Results and Discussion

GCHM is a brownish green mixture of different herbs with *vernonia amygdalina* as the main component. It had a characteristic bitter taste due to the saponin content characteristic of *vernonia amygdalina.* The pH of the bitters used was 4.1 ± 0.15, thus making it slightly acidic.

In-vitro dissolution study

The presence of the GCHM decreased the release of both Atz/r and Lpv/r in simulated intestinal fluid. At the 5-minute time interval, there was no significant difference in the release of Atz/r from Atz/r only formulation compared to Atz/r/GCHM. However, at the 30-minute time interval, 38.65% of Atz/r was released in the presence of GCHM when compared to 64.54% released with Atz/r. Atz/r release was significantly hindered by the presence of the polyherbal formulation as shown in Figure 1. Lpv/r release was also affected by the presence of the poly-herbal herbal bitters; this was likely due to increased pH of dissolution medium. In the presence of GCHM, Lpv/r release at 30 minutes was 24.37% compared to 39.43% when Lpv/r dissolution was carried out independently.

Clinical observations

The animals did not show any presence of piloerection, salivation and twitching during the duration of the experiment. This absence of observable signs of toxidromes indicated that in all the groups central nervous system involvement may not have occurred. There was however increased intake of feed and water by animals in groups IV, VI and VII. All the animals survived the duration of the experiment with animals in groups VI and VII showing 5.2 ± 1.7% and 5.5 ± 0.8% weight gain, respectively. All other groups did not show a significant increase in weight during the study period.

The effect of concomitant administration of GCHM and Lpv/r did not significantly affect biochemical parameters like total protein, globulin and total cholesterol ($p > 0.05$) (Table 1). ALT levels were significantly ($p < 0.05$) reduced when Lpv/r was given with GCHM, irrespective of the dose of GCHM given. Animals in groups V and VI which received Lpv/r/GCHM had ALT values of 27.19 U/L and 31.88 U/L, respectively whereas the control group had ALT value of 43.32 U/L. This reduction of ALT was also observed when GCHM was administered alone to the rats. This lowering of the values of the liver enzymes could indicate that the herbal cleanser contains active substances which could preserve liver cell membrane integrity. ALT values were however significantly elevated when Atz/r/GCMH was administered in group four 69.22U/L compared to the control 43.32 U/L ($p < 0.05$) (Table 2) and significantly decreased

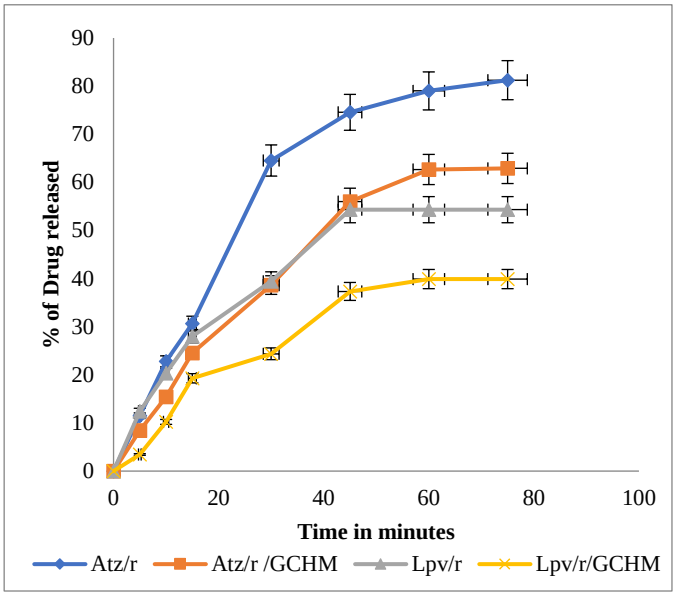

Figure 1: Dissolution profile of Atz/r, Atz/r/GCHM Lpv/r, Lpv/r/GCHM in simulated intestinal fluid pH 6.8.

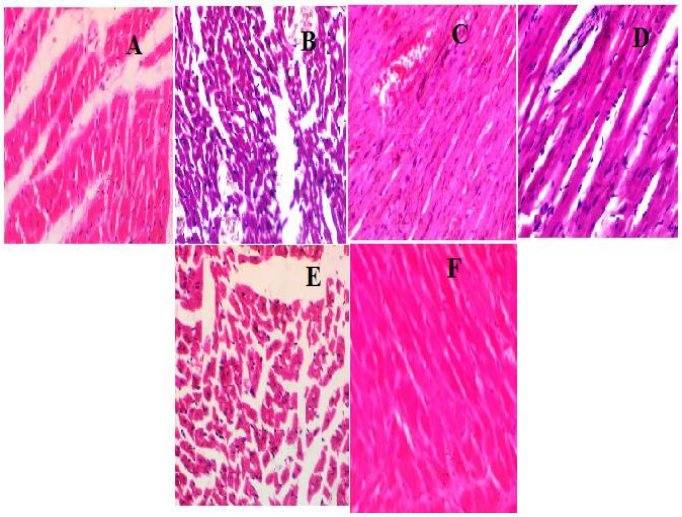

Figure 2: Photomicrograph of heart tissue. (A) control (B) GCHM (C) Atz/r (D) Lpv/r (E) Lpv/r/GCHM (1.5 mL/kg) (F) Atz/r/GCHM (1.5 mL/kg) depicting mild myocyte hypertrophy.

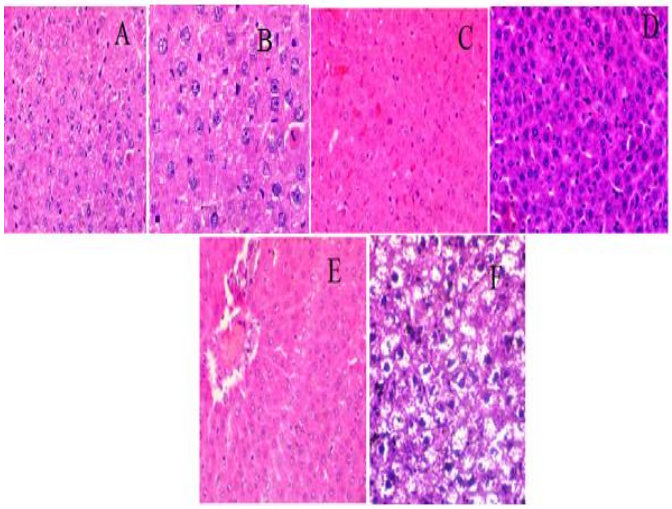

Figure 3: Photomicrograph of the liver (A) control (B) GCHM (C) Atz/r (D) Lpv/r (E) Lpv/r/GCHM (1.5 mL/kg) (F) Atz/r/GCHM (1.5 mL/kg) depicting hepatocytes showing feathery degeneration.

when Lpv/r/GCHM was administered 27.19 U/L (Table 1), ($p < 0.05$). This fluctuation of ALT values did not occur when GCHM was given alone, thus possible herb-drug interaction could be responsible for these elevated values which are an indication of hepatic tissue damage. There was a significant reduction in the value of creatinine levels when compared to the control 118.89 mmol/L ($p \leq 0.01$ when control was compared with Lpv/r/GCHM) with $p < 0.05$ at 87.55 mmol/L (Table 1). Globulin values were much higher in Lpv/r/GCHM group 42.17 g/L when compared to the control group 30.91 ($p \leq 0.05$), which is suggestive of conditions such as dehydration, allergies, liver disease and stress.[15]

Analysis of the haematological parameters indicated an increase in the WBC count 8.27×10^3µL and 7.82×10^3µL in groups V and VI compared to control 6.34×10^3µL ($p < 0.05$) as shown in Table 3 where GCHM and Lpv/r was concomitantly administered. HCT was slightly increased in group VI 56.1% compared to the control 44.37% ($p > 0.05$), these values, however, were not statistically significant. As shown in Table 4 there were no statistically significant variations in haematological parameters when Atz/r was co-administered with GCHM. Mean corpuscular volume (MCV) was reduced in group VI, (Lpv/r/GCHM) 62.7fl compared to control 73.85fl, MCV values in these groups were significantly different ($p < 0.05$) (Table 3). There was a slight elevation of MPV in group IV, 8.23 fl compared to control, 6.23 fl as well as a slight reduction in MCV in group III, 66.67 fl compared to the control, 73.85 fl ($p > 0.05$) was observed as in Table 4.

Gross examination of heart, lung, kidneys, brain and liver in all the treatment groups carried out did not show any morphological changes in the tissues examined. Photomicrographs of the heart tissue showed mild myocyte hypertrophy in group IV which received Atz/r/GCHM (Figure 2F). All other groups had normal histopathological features of the heart. In all the groups no damage to the kidneys was observed. Histopathological examinations of the photomicrographs of the liver hepatocytes showed feathery degeneration in group IV (Figure 3F), while the other groups had normal histopathological features of the liver.

This study assessed the short-term effect of concomitant use of marketed polyherbal mixture GCHM on the bioavailability of the protease inhibitors Lpv/r and Atz/r *in vitro*, while also assessing presence or absence of organ damage in rats. The presence of the herbal bitters was found to influence the amount of protease inhibitors available in the dissolution vessel. This may be due to the interaction of the components of these poly-herbal mixtures with the PIs.[10] Atz/r is usually administered with a light meal and it requires a pH mimicking intestinal pH for its absorption.[17] The presence of the bitters did not significantly reduce the amount of Atz/r released at 60 minutes 62.27%. The pH effect was more pronounced in the concomitant administration of GCHM with lopinavir (Lpv/r/GCHM) which significantly retarded the release of Lpv/r (Figure 1).

The concomitant administration of GCHM at high doses (1.5 mL/kg) with the protease inhibitors increased the WBC, hematocrit, Platelets, and MCHC, although, this increase was not statistically significant. Studies have shown that most patients with HIV, concurrently take their antiretrovirals with herbal medications. As such, the potential for clinically significant drug interactions between herbs and antiretrovirals is becoming increasingly appreciated.[9] Despite this awareness, little is known about the effect of commonly used herbal products such as herbal mixtures containing *Vernonia amygdalina* , *Saccharum officinarum*, *Allium sativum* , *Cajanus cajan* and *Zingiber officinale* on PIs dissolution and the overall effect on organ damage. Interacting herbal supplements have the potential to alter protease inhibitor plasma concentration, and has been shown with St John's wort and garlic. [5,10,11] Drug interactions may potentially increase ART plasma concentration, putting patients at risk of toxicity or lower drug concentration which may cause ART failure. Both Atz/r and Lpv/r rely on CYP3A4 metabolism for their elimination, in addition, both drugs are substrates for the transport protein p-glycoprotein which may also contribute to their distribution and elimination.

Table 1 showed that there was no statistically significant difference in the amount of total protein, albumin and globulin. The drug is bound to most of the plasma protein hence reduction in the amount of total protein. Reduction in total protein reflects liver disease or acute infection. Table 2 showed a significant elevation in liver enzymes ALT and AST ($p < 0.05$). The use of Atz/r over a long period of time causes elevation of liver enzymes and thus co-administration with a herbal mixture which causes increased liver enzyme levels is indicative of impending liver damage. This is expected as an increased challenge to the liver will lead to reduced enzyme production indicating liver damage. This could also be due to the leaking of these enzymes from the liver cytosol to the bloodstream, indicating also that the high dose of the herbal mixture is hepatotoxic.

Table 1: Effect of concomitant administration of GCHM and Lopinavir on biochemical parameters of Wistar rat plasma after a 15-day treatment.

Parameter	Control (DW)	GCHM (1.5 mL/kg)	Lpv/r	Lpv/r/GCHM (1.5 mL/kg)	Lpv/r/GCHM (0.5 mL/kg)	P value
Total Protein (g/L)	71.55 ± 7.05	73.55 ± 2.04	66.23 ± 4.09	84.46 ± 1.36	80.51 ± 1.39	0.09
Albumin (g/L)	42.09 ± 1.16	41.54 ± 0.93	40.77 ± 0.55	42.29 ± 2.06	43.22 ± 4.82	0.42
Globulin (g/L)	30.91 ± 7.11	39.99 ± 0.23	28.94 ±4.07	42.17 ± 0.74*	37.68 ± 5.41	0.05
ALT (U/L)	43.32 ± 2.73	32.73 ±2.11	43.89 ± 3.02	27.19 ± 1.56*	31.88 ± 1.05	0.04
AST (U/L)	142.95 ± 0.23	128.1 ±0.72	146.97 ± 3.26	96.68 ± 5.37	95.14 ± 3.56	0.05
Total Cholesterol	2.26 ± 0.23	2.42 ±0.11	2.34 ± 0.41	2.07 ± 0.15	2.51 ± 0.37	0.31
Urea (mmol/L)	8.58 ± 0.06	7.99 ± 0.19	8.52 ± 0.22	7.64 ± 0.31	7.69 ± 0.32	0.12
Creatinine (mmol/L)	118.89 ± 5.77	103.38 ± 1.01	121.30 ± 1.56	87.55 ± 3.97*	88.26 ± 4.20	0.03

Values are expressed as means ± SD, (n = 10), $p \leq 0.05$ were considered as statistically significant. * $p \leq 0.01$ when control was compared with Lpv/r/GCHM.

Table 2: Effect of concomitant administration of GCHM and Atazanavir on biochemical parameters of Wistar rat plasma after a 15-day treatment

Parameter	Control (DW)	GCHM (1.5 mL/kg)	Atz/r	Atz/r/GCHM (1.5 mL/kg)	Atz/r/GCHM (0.5 mL/kg)	P value
Total Protein (g/L)	71.55 ± 7.05	73.55 ± 2.04	70.49 ± 2.39	58.17 ± 4.47	60.93 ± 0.86	0.06
Albumin (g/L)	42.09 ± 1.16	41.54 ± 0.93	41.76 ± 1.45	44.60 ± 1.14	40.79 ± 0.60	0.11
Globulin (g/L)	30.91 ± 7.11	39.99 ± 0.23	28.75 ± 1.53	35.57 ± 3.33	28.14 ± 0.74	0.12
ALT (U/L)	43.32 ± 2.73	32.73 ±2.11	45.95 ± 4.37	69.22 ± 0.68*	62.55 ± 6.70	0.04
AST (U/L)	142.95 ± 0.23	128.1 ±0.72	145.69 ± 4.92	184.66 ± 13.38	170 ± 1.08	0.06
Total Cholesterol	2.26 ± 0.23	2.42 ±0.11	2.44 ± 0.33	2.52 ± 0.46	2.35 ± 0.35	0.75
Urea (mmol/L)	8.58 ± 0.06	7.99 ± 0.19	8.91 ± 0.77	9.05 ± 0.28	8.99 ± 0.61	0.83
Creatinine (mmol/L)	118.89 ± 5.77	93.38 ± 1.01	125.11 ± 5.43	100.41 ± 14.55	110.12 ± 7.33	0.80

Values are expressed as means ± SD, (n = 10), $p \leq 0.05$ were considered as statistically significant. * $p \leq 0.01$ when control was compared with Atz/r/GCHM.

Table 3: Effect of concomitant administration of GCHM and Lopinavir on haematological parameters of Wistar rat plasma after a 15-day treatment.

Parameter	Control (DW)	GCHM (1.5 mL/kg)	Lpv/r	Lpv/r/GCHM (1.5 mL/kg)	Lpv/r/GCHM (0.5 mL/kg)	P value
WBC (10^3µL)	6.34 ± 0.314	6.85 ± 0.33	6.11 ± 0.22	8.27 ± 0.16	7.82 ± 0.61	0.08
LYM (10^3µL)	5.84 ± 0.13	6.01 ± 0.21	5.95 ± 0.07	6.19 ± 0.11	6.35 ± 0.09	0.13
RBC (10^6 µL)	7.31 ± 0.19	7.40 ± 0.43	7.44 ± 0.38	7.44 ± 0.13	7.39 ± 0.22	0.33
HGB (g/dl)	13.29 ± 0.37	13.88 ± 0.92	12.51 ± 0.19	13.21 ± 0.23	12.29 ± 0.18	0.18
HCT (%)	44.37 ± 4.44	45.01 ± 0.99	43.18 ± 3.59	56.10 ± 1.64	44.81 ± 3.02	0.73
MCV (fl)	73.85 ± 1.55	72.99 ± 0.43	73.63 ± 0.79	62. 70 ± 2.44*	63.39 ± 2.18	0.04
MCH (Hg)	17.89 ± 0.78	17.99 ± 0.82	17.78 ± 0.38	17.52 ± 0.41	17.93 ± 0.21	0.56
MCHC (g/Dl)	30.58 ± 0.72	28.93 ± 0.41	30.12 ± 1.09	37.80 ± 1.41	29.16 ± 0.62	0.92
RDW –c (%)	14.14 ± 0.56	14.87 ± 0.21	13.51 ± 0.52	13.50 ± 0.15	14.96 ± 0.86	0.83
PLT (10^3/ µL)	704.36 ± 6.95	755.22 ± 4.32	648.25 ± 34.52	756.74 ± 40.87	661.73 ± 12.47	0.09
MPV (fl)	6.23 ± 0.23	6.84 ± 0.11	6.23 ± 0.21	8 .00 ± 0.66	6.60 ± 0.35	0.21
PDWc (fl)	7.74 ± 0.29	7.93 ± 0.05	7.35 ± 0.19	7.69 ± 0.36	7.92 ± 0.31	0.33
PCT	0.31 ± 0.03	0.20 ± 0.09	0.34 ± 0.04	0.17 ± 0.02	0.26 ± 0.06	0.42

Values are expressed as means ± SD, (n = 10), $p \leq 0.05$ were considered as statistically significant. * $p \leq 0.01$ when control was compared with Lpv/r/GCHM.

Table 4: Effect of concomitant administration of GCHM and Atazanavir on haematological parameters of Wistar rat plasma after a 15-day treatment.

Parameter	Control (DW)	GCHM (1.5 mL/kg)	Atz/r	Atz/r/GCHM (1.5 mL/kg)	Atz/r/GCHM (0.5 mL/kg)	P value
WBC (10^3µL)	6.34 ± 0.314	6.85 ± 0.33	6.32 ±0.37	7.96 ± 0.84	6.62 ± 0.33	0.93
LYM (10^3µL)	5.84 ± 0.13	6.01 ± 0.21	5.91 ± 0.80	6.22 ± 0.19	5.36 ± 0.19	0.08
RBC (10^6 µL)	7.31 ± 0.19	7.40 ± 0.43	7.55 ± 0.29	7.66 ± 0.29	7.26 ± 0.70	0.37
HGB (g/dl)	13.29 ± 0.37	13.88 ± 0.92	13.29 ± 0.86	13.78 ± 0.69	12.91 ± 0.94	0.07
HCT (%)	44.37 ± 4.44	45.01 ± 0.99	44.65 ± 1.97	51.64 ± 2.46	43.6 ± 3.03	0.52
MCV (fl)	73.85 ± 1.55	72.99 ± 0.43	73.86 ± 2.67	74.13 ± 3.57	66.07 ± 4.56	0.09
MCH (Hg)	17.89 ± 0.78	17.99 ± 0.82	18.35 ± 0.37	18.74 ± 0.79	18.03 ± 0.12	0.11
MCHC (g/Dl)	30.58 ± 0.72	28.93 ± 0.41	30.67 ± 1.90	37.25 ± 2.80	28.39 ± 0.70	0.83
RDW –c (%)	14.14 ± 0.56	14.87 ± 0.21	14.76 ±1.16	14.58 ± 2.37	13.50 ± 0.31	0.07
PLT (10^3/ µL)	704.36 ± 6.95	755.22 ± 4.32	749.26 ± 65.17	744.45 ± 49.51	654.01 ± 55.05	0.73
MPV (fl)	6.23 ± 0.23	6.84 ± 0.11	6.60 ± 0.26	8.23 ± 0.15	6.17 ± 0.67	0.62
PDWc (fl)	7.74 ± 0.29	7.93 ± 0.05	7.60 ± 0.36	7.62 ± 0.34	7.65 ± 0.25	0.33
PCT	0.31 ± 0.03	0.20 ± 0.09	0.31 ± 0.32	0.26 ± 0.12	0.32 ± 0.21	0.71

Values are expressed as means ± SD, (n=10), $p \leq 0.05$ were considered as statistically significant.

This was consistent with decreased levels of total protein and albumin also indicative of liver damage. The histology of the liver in Atz/r/GCHM (1.5 mL/kg) depicting hepatocytes showing feathery degeneration in Figure 3F is also indicative of liver damage where Atz/r/GCHM (1.5 mL/kg) is co-administered.

The photomicrograph of the heart tissue showed that in animals that were treated with Atz/r/GCHM (1.5 mL/kg) their heart tissue exhibited myocyte degeneration (Figure 2F). This is a critical factor especially for patients who are on antihypertensive agents and antiretrovirals and also consume these bitters indiscriminately. This also is a strong indication that the contents of the herbal preparation contained strong heart muscle degenerating substances, which could lead to vascular eruption, loss of blood, and death.

Allium sativa, one of the components of GCHM has been found to reduce cholesterol [22] and hence muscle mass in the body and so the high dose of the GCHM may lead to a reduced creatinine concentration since creatinine is being produced from muscle mass, and transported through the kidney. This also indicates that the contents of GCHM possess antilipidemic agents, with potentials of reducing the cholesterol levels. The red blood cell count, haemoglobin and hematocrit were also increased with high doses of the herbal mixture, although the difference was not statistically significant, the numerical increase was observed in the RBC, HCT, HGB values, indicating that the content of GCHM contains hematopoietic not hematotoxic substances. The high dose GCHM was found to induce an elevated platelet count, this should be expected as *Vernonia amygdalina* being a main component of the herbal mixture has been shown to contain hematostatic substance.[23] Traditional herbal use has been reported to be common among individuals with moderate and advanced HIV disease,[9] some of these herbal medicines and herbal mixtures do not pose a health threat to the individuals consuming them as long as they are not taken together with conventional medicine.[9, 12, 20] In Africa, traditional herbal medicines containing *Saccharum officinarum*, *Allium sativum*, *Cajanus cajan* and *Zingiber officinale* often used as primary treatment for HIV/AIDS and for management of opportunistic infections including dermatological disorders, nausea, depression, insomnia and weakness[5]. They are not dose regulated, hence the huge disparities between the doses the patients take. [23, 24] Thus the need for enlightenment to dissuade patients from using orthodox medicines concomitantly with herbal drugs. This study showed that the presence of GCHM in dissolution media altered the release of both protease inhibitors studied leading to a reduced release of Atz/r and Lpv/r in the dissolution media, thus altering pharmaceutical availability. Organ damage was observed only when the high dose of the herbal mixture was taken with Atz/r in Wistar rats. Damage to the hepatic tissue as well as to cardiac tissue was observed after daily dosing of GCHM and Atz/r concomitantly. When GCHM was however administered alone to the rats there were no statistically significant changes in the biochemical, haematological and pathological parameters of the animals assessed. This would suggest that administration of GCHM alone is not likely to cause harmful effects in humans, the concomitant administration of GCHM at a high dose with Atz/r is likely to cause organ damage.

Conclusion

Concomitant administration of marketed poly-herbal formulation GCHM, alongside PIs, adversely affects pharmaceutical availability which may influence the bioavailability of the PIs and may cause hepatotoxicity. Interactions based on the inhibition/induction of metabolic enzymes will need to be studied utilizing GCHM and PIs.

Conflict of interest

The authors declare no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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