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In vitro screening of NIPRD-AH1 on CYP3A4 activity for plausible herb-drug interaction

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ABSTRACT

NIPRD-AH1 is being developed from freeze-dried aqueous extract of *Andrographis paniculata* at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria as immune-stimulant for HIV infected patients. The aim of this study was to evaluate the effect of NIPRD-AH1 on human cytochrome P450 3A4 (CYP3A4) drug metabolising enzyme in order to generate clinically significant data for its safe and efficacious use. Activity on CYP3A4 was measured with and without the addition of NIPRD-AH1 in a reaction medium with testosterone (70 µM) as CYP3A4 substrate, and ketoconazole (2.5 µM) as positive inhibitor. The metabolites formed after the enzymatic reactions were quantified by validated HPLC techniques. Results showed NIPRD-AH1 exhibiting low IC_{50} value of 0.03 mg/ml, indicating that its metabolic processes are likely to inhibit CYP3A4. This suggests possibility of herb-drug interaction, with potential implication on concomitant administration of NIPRD-AH1 with CYP3A4 substrates. We therefore suggested that this effect be examined *in vivo* in order to draw a definitive conclusion.

Keywords: *Andrographis paniculata*, NIPRD-AH1, CYP3A4, herb-drug interaction.

INTRODUCTION

Andrographis paniculata Nees [Acanthaceae] -- Pl. Asiat. Rar. (Wallich). iii. 116. (IK) (IPNI, 2012) is a bitter annual herb, native to tropical Asian countries, and is distributed in other regions of the world (Farnsworth and Soejarto, 1991). The plant is reputed for its medicinal value, necessitating a wide array of studies conducted on it by researchers. Results from these studies show that the plant exhibited various biological efficacies in both experimental and clinical studies, and also contained useful phytochemicals; detailed in the recent review

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by Okhuarobo et al. (2014). The plant is domesticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria with a seed obtained from India in the 1990s. Its use was popular among HIV patients under the name Conavir before anti-HIV drugs became readily available (Ameh et al., 2010). The aqueous freeze-dried leaves extract of *Andrographis paniculata* (NIPRD-AH1) is being developed at NIPRD as immune-stimulant for Human Immune Virus (HIV) infected patients.

The use of herbal medicines either alone or with antiretroviral (ARVs) therapy is widespread among patients living with HIV (Duggan et al., 2001). This should be of concern especially with evidences of pharmacokinetic interactions between herbal products and ARVs (Lamorde et al., 2012). However, of recent, there has been growing awareness of herb-drug interaction as herbal remedies usage became popular. Pharmacology and toxicology research had been advocated on such herbal medications to ensure validity, safety and efficacy (Calapai and Caputi, 2007). Therefore, there is the need to make scientific evidences on these herbal products available for users and caregivers. Much attention is being given to herbal medicines interactions with cytochrome (CYP) 450 drug metabolizing enzymes. Of its many subtypes, one of the most important isoform is CYP3A4 (Nerbert and Russell, 2002), being involved in the metabolism of majority of clinically used drugs. For this reason, CYP3A4 is recognized as a major candidate for pharmacokinetic interactions (Schrøder-Aasen et al., 2012). Interestingly, most of the ARVs currently approved for use are metabolized in the liver by CYP3A4 (PAGAA, 2014). Previous studies with extracts of *Andrographis paniculata* on these enzymes shows that the extracts effectuate mouse hepatic cytochrome P450 enzymes (Jarukamjorn et al., 2006); and inhibited human hepatic CYP2C19 (Pan et al., 2011). The objective of this study is to assess potential or otherwise of clinical drug interaction by testing the effect of NIPRD-AH1 on human hepatic CYP3A4 in order to generate useful data for guidance towards its safe and efficacious use (Adzu et al., 2013).

MATERIALS AND METHODS

Herbal product (NIPRD-AH1)

NIPRD-AH1 preparations, standardisation, physicochemical and other quality control variables have been reported (Ameh et al., 2010). Briefly, aerial parts of *Andrographis paniculata* (voucher number NIPRD/H/3720) were harvested and subsequently air-dried under shade, grounded into powder, extracted with distilled water, filtered and freeze-dried. The freeze dried extract was evaluated to ensure its compliance with an already established standard and samples were delivered to the African Institute of Biomedical Science and Technology (AiBST), Harare, Zimbabwe in January 2010 for the enzyme assay. NIPRD-AH's limit of solubility in aqueous solution was estimated by turbidimetric assay on plate reader with absorbance monitored at 595 nm.

CYP3A4 enzyme assay

Effect of NIPRD-AH1 on CYP3A4 (Cypex Ltd, UK) activity was first investigated at 2 concentrations; low (0.02 mg/ml) and high (2 mg/ml). Inhibition of the CYP activity by more than 20% was considered significant. In this case, the IC_{50} value was determined using 8 concentrations range (0.001 – 2 mg/ml) of NIPRD-AH1 and the data analysed using nonlinear regression.

The incubation was performed at 37 ^{o}C in reactive mixtures with a final volume of 300 µL, consisting of: 5 µL of 0.5 mg/ml CYP3A4, 20 µL of 0.1M pH 7.4 KPO_4 buffer, 20 µL of $MgCl_2$, 5 µL of 70 µM testosterone (Sigma-Aldrich, St. Louis, MO), and 135 µL of water, with and without the addition of 4 µL of NIPRD-AH1 or 2.5 µM Ketoconazole (positive control). The incubation was started by 10 µL of the marker substrate (1 mM NADPH) and stopped after 15 min with 100 µL of ice cold methanol (Adzu et al., 2014).

The mixture was centrifuged (at 10,000 g) for 10 min at 4 ^{o}C, and the supernatant transfer to High Performance Liquid Chromatography (HPLC) vials for analysis. All working solutions were freshly prepared before each experiment.

HPLC analysis

The analytical procedure was performed using validated HPLC methodology with Agilent HPLC system coupled with a UV detector under the following conditions: mobile phase, solvent A: 60% methanol; solvent B: 40% 50 mM KH_2PO4; flow rate of 1ml/min; injection volume of 100 µL; analytical column: C18, 4.6 x 150 mm, 5 µM; detection wavelength of 254 nm and run time of 19 min at room temperature (Adzu et al., 2014).

RESULTS AND DISCUSSION

In vitro assays such as this are the most practical means of screening for potential interaction for herbal products. The solubility test shows that NIPRD-AH1 has solubility above 2 mg/ml in water, indicating that it was soluble at that concentration being the highest concentration that was used in the inhibition assay. The enzyme assay showed that NIPRD-AH1 inhibited CYP3A4 activity by more than 50%, with IC_{50} value of 0.03 mg/ml. The IC_{50} profile is presented in Figure 1.

If CYP activity is altered-so too is the bioavailability of drugs that are metabolised by it. The way herbal products alter the action of CYP3A4 is by inhibition, causing toxicity in most cases (Lau et al., 2013). However, many phytodrugs have similar activity, warranting further studies *in vivo* to determine whether the interactions are of significant issue. This is because such *in vitro* observation might not necessarily result in major interaction *in vivo*. There is the possibility of other effects; for example, solubility and permeability parameters and other extraction and manufacturing process (Lipinski et al., 1997).

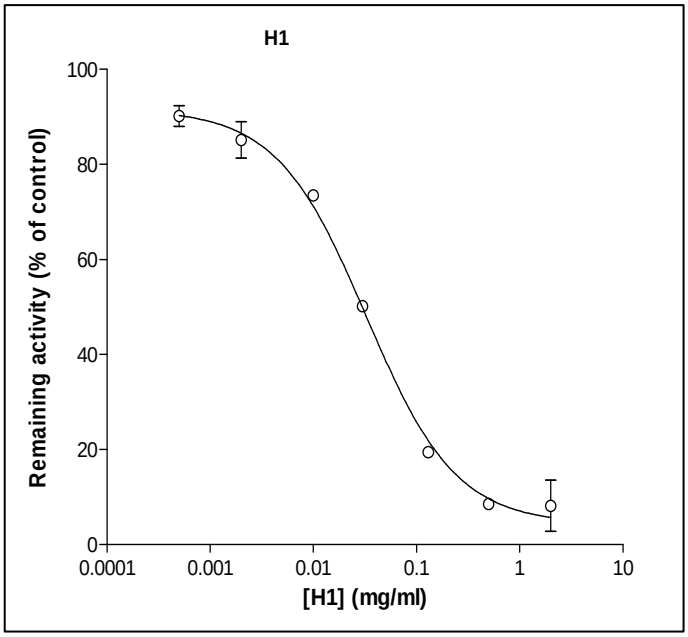

Figure 1: Effect of NIPRD-AH1 on CYP3A4 activity.

Conclusion

This *in vitro* result shows that NIPRD-AH1 is a potential inhibitor of CYY3A4. Therefore, its clinical relevance needs to be assessed *in vivo* to put it in proper prospective (FDA, 2012). Equally, the fact that inhibition of CYP3A4 has been exploited in some cases for its potential clinical benefits (Lee et al., 2007) can also be explored.

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