

Original Article

INVESTIGATION OF CYP1A INTERACTION POTENTIAL OF *WITHANIA SOMNIFERA* IN RAT AND HUMAN LIVER MICROSOMES

JAY SAVAI,^{1*} NANCY PANDITA², MEENA CHINTAMANENI¹

¹Department of Pharmacology, Shobhaben Pratapbhai Patel, School of Pharmacy and Technology Management, SVKM's NMIMS, Vile Parle (W), Mumbai-400056, India, ²Department of Phytochemistry, School of Science, SVKM's NMIMS, Vile Parle (W), Mumbai-400056, India.
Email: jaysavai08@gmail.com

Received: 21 Nov 2014 Revised and Accepted: 15 Dec 2014

ABSTRACT

Objective: The aim of this study was to investigate CYP1A interaction of crude extracts (ethanolic, methanolic, hydromethanolic & aqueous) of *Withania somnifera* and its principal phytoconstituents (withaferin-A and withanolide-A) in rat and human liver microsomes.

Methods: *In vitro* study were carried out in both rat & human liver microsomes while, *in vivo* CYP1A interaction potential was investigated by administering the methanolic extract of *Withania somnifera* orally at a dose of 500 mg/kg in male Wistar rats using probe substrate technique.

Results: The results of this study revealed that IC₅₀ values of all the crude extracts of *Withania somnifera* and its principal phytoconstituents (withaferin-A & withanolide-A) were found to be >640 µg/ml and >32 µM respectively, for CYP1A enzyme both in rats and humans. However, *in vivo* pharmacokinetic study of co-administered methanolic extract of *Withania somnifera* and phenacetin, revealed that the crude extract lead to an approximate 1.5 fold (31%) decrease in AUC_{0-24h} (p < 0.05). Elimination rate constant (Ke) increased by 2 fold (48%) and half-life (T_{1/2}) decreased by 1.8 fold (43%).

Conclusion: The results of this study suggested that *Withania somnifera* showed no *in vitro* CYP1A inhibition in both rats and humans. However, *in vivo* administration of methanolic extract of *Withania somnifera* significantly induced CYP1A enzyme & subsequently altered the pharmacokinetics profile of phenacetin in rats; indicating a potential for herb-drug interactions.

Keywords: *Withania somnifera*, CYP1A interaction, Phenacetin, Human liver microsomes, Pharmacokinetics, Herb-drug interaction.

INTRODUCTION

The most versatile enzyme system involved in the metabolism of xenobiotics is cytochrome P₄₅₀. The cytochrome P₄₅₀ enzymes (CYP) represent a large family of proteins involved in the metabolism of drugs and other xenobiotics, as well as some endogenous substrates. The major drug-metabolizing enzyme (DME), cytochrome P₄₅₀ (CYP), consists of the superfamily of heme proteins that catalyze the oxidative metabolism of a wide variety of exogenous chemicals including drugs. Several isoforms, such as CYP1A2, CYP2C9, CYP2D6 and CYP3A4 appear to be the most relevant isozymes involved in the metabolism of clinically significant drugs. Inhibition of these enzymes often results in unexpected and sometimes severe adverse drug interactions, as the metabolic clearance of co-administered drugs can be altered dramatically [1].

Drug interactions can frequently arise when drugs are co-administered and one drug inhibits the metabolic clearance of the second drug by inhibition of a specific CYP enzyme. Inhibition of CYP enzymes can also be affected by natural products. A notable example of this is the inhibition of CYP3A by grapefruit juice, which can result in elevations of systemic exposure to CYP3A-cleared compounds [2]. The use of herbs as alternative and complementary therapy has increased worldwide. Patients taking herbs together with prescribed western medication are at a potential risk of herb-drug interactions. Some patients self-medicate with several different herbs and herbal preparations without their doctor's recommendation [3].

Herb-drug interaction (HDI) is one of the most important clinical concerns due to concomitant consumption of herbs and prescription drugs. The necessity of poly-pharmacy in the management of most diseases further increases the risk of HDI in patients. The ability of intestinal and hepatic CYP to metabolize numerous structurally unrelated compounds, apart from being responsible for the poor oral bioavailability of numerous drugs is responsible for the large number of documented drug-drug and drug-herb interactions [4]. One of the main tasks of some of the CYP₄₅₀ enzymes is the metabolism of drugs. The actions of human cytochrome P₄₅₀ 1A2 may partly account for the carcinogenic effects of burned foods and

cigarette smoke. Burning these substances alters amino acids and carbohydrates, producing heterocyclic amines. Human cytochrome P₄₅₀ 1A2 (CYP1A2), a member of the cytochrome P₄₅₀ mixed function oxidase system, is one of the important enzymes involved in the metabolism of xenobiotics in the body. CYP1A2 constitutes 13% of the total CYP content in the liver and plays an important role in the metabolic clearance of approximately 5% of currently marketed drugs. Many studies have reported inhibition data on CYP1A2 by many different herbs. For example, St John's wort, the components of *Ginkgo biloba* and flavonoids extracted from plants could decrease the activation of CYP1A2 [3,5].

Withania somnifera (L.) Dunal (Solanaceae) also known as ashwagandha, Indian ginseng and Winter cherry is a well-known herb which has been traditionally used to treat various symptoms and conditions, It is an adaptogen mainly used for its action against chronic stress. It also shows certain potential therapeutic activities like anti-inflammatory, anti-tumor, anti-oxidant and antibacterial. *Withania somnifera* Dunal (WS) has been used for centuries in ayurvedic medicine to increase longevity and vitality [6]. Western research supports its poly-pharmaceutical use, confirming antioxidant, anti-inflammatory, immune-modulating, and anti-stress properties in the whole plant extract and several separate constituents [7]. As an antioxidant, WS and active constituents, sitoindosides VII-X and withaferin-A (WA), withanolide-A (WL-A) and withanoside I-IV have been proven to increase levels of endogenous superoxide dismutase, catalase, and ascorbic acid, while decreasing lipid peroxidation [8-10]. It also shows certain potential therapeutic activities like anti-tumor and antibacterial [11-13]. The structure of principal phytoconstituents of *Withania somnifera*, withaferin-A & withanolide-A is represented in fig. 1. Thus, ash wagandha is commonly prescribed clinically for its anti-inflammatory, antistress, immunomodulatory and adaptogenic properties and is most widely consumed herb. Additionally, its phytoconstituents may be inducers, inhibitors and/or substrates of CYP1A enzyme, thus could lead to serious herb-drug interactions. Therefore, in the present research work, we have investigated the *in vitro* and *in vivo* interaction potential of *Withania somnifera* with

cytochrome 1A (CYP1A) to understand the possible herb-drug interaction. The CYP1A inhibition potential was evaluated using Rat (RLM) and Human liver microsomes (HLM) by *in vitro* studies and the CYP1A induction potential was evaluated by *in vivo* pharmacokinetics studies in rats. CYP1A inhibition or induction potential of the herb (WS) as a whole and/or its phytoconstituents (withaferin-A & withanolide-A) will be an indicator of a probable herb-drug interaction, with other allopathic drugs that are primarily metabolized by CYP1A enzyme.

MATERIALS AND METHODS

Chemicals and equipment

All the solvents, chemicals and reagents used were of analytical grade and purchased locally. Phenacetin, paracetamol and caffeine were purchased from Sigma-Aldrich Ltd. Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPH) was purchased from SRL Labs Pvt. Ltd. HPLC grade acetonitrile was purchased from Thermo Fischer Scientific India Pvt. Ltd. Withaferin-A & withanolide-A were gift samples from Natural Remedies, Bangalore, India. Pooled human liver microsomes (HLM) of twenty individual male donors (Batch no. 0710112) were purchased from Krishgen Biosystems. RP-HPLC system consisted of a Shimadzu LC 2010, with an auto sampler, PDA detector using LC Solutions @ software

Experimental animals

Male Swiss Wistar albino rats were selected for the *in vitro* and *in vivo* pharmacokinetics study. They were housed under standard conditions ($24 \pm 2^\circ\text{C}$; relative humidity 60-70 %) for a week. The *in vitro* and *in vivo* experiment was performed as per the guidelines of Institutional Animal Care Committee constituted as per the guidelines of the CPCSEA and the protocol [Protocol no. CPCSEA/IAEC/SPTM/P-60/2014] was duly approved by the Institutional Animal Ethics Committee.

Extraction and preparation of test samples

Fresh roots of *Withania somnifera* (WS) were purchased from Zandu Foundation, Gujarat, India. Roots were identified by Dr. Naik, Senior Research Scientist, Piramal Life Sciences and authenticated by Agharkar Research Institute, Pune with the voucher specimen (R-128) deposited for further reference. Fresh roots were dried, powdered and extraction was carried out by cold maceration technique. Approximately, 500 gm of dried powdered root of *W. somnifera* was defatted initially with around 2 liters of petroleum ether AR (60-80°C) for 2 days with intermittent shaking. This was followed by extraction by cold maceration technique using 2 litres of the respective solvents like methanol, hydromethanol (water and methanol in equal ratio), absolute ethanol and water, individually to yield methanolic, hydromethanolic, ethanolic and aqueous extracts. Alcoholic and hydroalcoholic extracts were evaporated to dryness using rotary vacuum evaporator while aqueous extracts were dried by lyophilization. Further, all the extracts collected, were dried & stored in vacuum desiccator till use. Stock solutions of methanolic, hydromethanolic, ethanolic and aqueous extracts of *W. somnifera* were prepared in methanol, hydromethanol (water and methanol in equal ratio), ethanol & dimethyl sulfoxide (DMSO), respectively to yield solutions of final concentration 5 mg/mL. Stock solutions of phytoconstituents (withaferin-A & withanolide-A) were prepared in methanol to yield solutions of final concentration 10 mM. Stock solutions of probe substrate, phenacetin was prepared in methanol (12 & 30 mM) while phosphate buffer (100 mM, pH 7.4) was used to make NADPH solution (10 mM).

Quantification of phytoconstituents using RP-HPLC analysis

Percent w/w content of WA & WL-A in all the four crude extracts of WS was quantified using RP-HPLC. WA & WL-A were used as a reference standard, dissolved in methanol to yield a stock solution of 1 mg/ml which was subsequently diluted to prepare working standard solutions with concentrations in the range of 10–100 µg/ml. Accurately weighed crude extracts (30mg/ml) were sonicated with respective solvents mentioned above and filtered through Whatman NYL 0.45 µ syringe filter. A Kromasil C₁₈ column

(250 mm×4.6 mm, 5 µm particle size) was used as stationary phase in the RP-HPLC system. Simultaneous quantification of withaferin-A and withanolide-A in crude extracts of *W. somnifera* was carried out using a validated method reported by Chaurasia et al. 2008 [14]. The phytoconstituents were quantified at 227 nm and the retention time of withaferin-A & withanolide-A was found to be 9.2 and 16.6 mins, respectively. The analysis was repeated for three times (n=3) by comparing and interpolating the extract peak area (response) with that of the standard WA & WL-A from the calibration curve.

Preparation of Rat liver microsomes

Rat liver microsomes were isolated from male Swiss Wistar (150-200 gm) strain rats based on the methods described by the calcium aggregation method [15]. Briefly, the rats were euthanized by cervical dislocation and the livers (20 gm) were quickly removed, perfused with 1.15% potassium chloride (KCl) solution and homogenized with four volumes (80 ml) of ice cold 10 mM Tris-HCl buffer containing 0.25M Sucrose, pH 7.4, in a Potter glass homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at 13,000 × g for 10 min at 4°C in a refrigerated centrifuge (Eppendorff) and the precipitate was discarded. To the supernatant, calcium chloride was added to yield a final concentration of 10 mM. The solution was stirred for 15-20 min, and then centrifuged at 25,000 × g for 10 min at 4°C. The firmly packed pellets of microsomes were re suspended by homogenization in 100 mM Tris-HCl buffer containing 20% w/v glycerol and 10 mM EDTA, pH 7.4. The microsomes were stored at -80°C until use. Protein concentrations were determined by Lowry et al. [16] using bovine serum albumin as standard. The optical density was recorded on a Perkin Elmer UV/vis spectrophotometer at 625 nm.

CYP1A inhibition assay

Microsomes are a valuable tool for investigating the metabolism of compounds (enzyme inhibition, clearance and metabolite identification) and for examining drug-drug interactions by *in vitro* study. *In vitro* studies can identify metabolites, species-specific metabolic routes, and the experimental animal model that best reflects the potential human exposure to the drug and its metabolites. This information can also be useful in the design of the clinical studies by identifying human metabolites, the enzymes responsible for the metabolic clearance of the drug, the effects of genetics and other host factors on the metabolism of the drug, and potential drug-drug interactions [17]. Rat and Human liver microsomes were used for assessing the inhibition potential of crude extracts of *Withania somnifera* (10-640 µg/ml) and WA & WL-A (1-32 µM) by estimating phenacetin o-de ethylation activity and the inhibition potential was compared with the positive control α-naphthoflavone (known CYP1A inhibitor in rats and humans). α-naphthoflavone was incubated at a concentration of 0.1 µM in HLM and 30 µM in RLM which inhibited phenacetin metabolism by 50%. Briefly, a standard 100 µl incubation mixture contained liver microsomes (1mg/ml protein concentration), phenacetin {Km: 54 µM in RLM [18] and 150 µM [19] in HLM} in 0.1 M sodium phosphate buffer pH 7.4 at 37°C was incubated for 1h, in triplicate. The reactions were initiated with NADPH (final concentration 1 mM) and then terminated with 50 µl of internal standard caffeine (50 µg/ml) in methanol. The samples were centrifuged at 4000 rpm for 10 mins at 4°C and the supernatant were subjected to RP-HPLC analysis. Modulatory effects of crude extracts of WS & its principal phytoconstituents (WA and WL-A) were evaluated by incubation of rat and human liver microsomes, phenacetin with or without herbal compounds. Solutions of different concentration of crude extract of WS were prepared in the respective solvents & its principal phytoconstituents (WA and WL-A) were prepared in methanol. Negative control incubations with respective solvents and positive control incubations with α-naphthoflavone were run simultaneously. In all the incubations organic content was not more than 1%v/v. The formation of paracetamol was subsequently quantified using RP-HPLC. Samples were run on a C₁₈ column and mobile phase used was (A) Buffer (0.1M Ammonium acetate buffer pH=5) & (B) methanol and was pumped at a flow rate of 1 ml/min. The gradient program used was time: %B - 0 /10; 5/80; 10/80; 12/10; 15/10. Detection of paracetamol and phenacetin was

accomplished by UV absorbance at a wavelength of 240 nm. Retention times for paracetamol, caffeine and phenacetin were 5.7, 6.6 and 8.1 mins, respectively. Percentage inhibition of crude extracts of WS & its principal phytoconstituents (WA and WL-A) to inhibit de-ethylation of phenacetin was calculated by the formula mentioned below:

$$\% \text{ inhibition} = \left\{ \frac{(\text{AR control} - \text{AR sample})}{\text{AR control}} \right\} \times 100$$

AR control = Area ratio of paracetamol/caffeine in negative control (solvent)

AR sample = Area ratio of paracetamol/caffeine in presence of crude extracts of WS & its principal phytoconstituents (WA and WL-A)

IC₅₀ values were calculated using Graph Pad Prism®.

In vivo Pharmacokinetic (PK) study design

Male Wistar rats were randomly divided into two groups (n=6). Group I was administered orally with 0.5 % sodium carboxymethyl cellulose for 10 days. Group II was orally administered with methanolic extract of *Withania somnifera* (500mg/kg suspended in 0.5% sodium carboxymethyl cellulose) [20] for 10 days. Twenty-four hours after the last dose, animals of Group I & II were administered with a single oral dose of Phenacetin (200mg/kg suspended in 0.5% sodium carboxymethyl cellulose). Whole blood samples (500µl) were withdrawn from the retro orbital sinus at 5, 15, 30, 60, 120, 240, 360 min and 24h after phenacetin

administration. Disodium EDTA was used as the anticoagulant. The blood samples were centrifuged at 4000 rpm for 10 mins at 4°C and plasma was separated and stored at -20°C until RP-HPLC analysis was carried out.

Plasma sample preparation and analysis

To a 100 µL plasma sample, 10 µL of caffeine (internal standard) was spiked. Samples were then vortex-mixed for 1-2 mins and extracted with 1 ml ethyl acetate by vortex-mixing for 5 mins. Liquid-liquid extraction technique using ethyl acetate for extraction was followed, since ethyl acetate gave maximum percent recovery of phenacetin, as compared to the other solvents tried. After centrifugation at 4000 rpm, 4°C for 10 mins, the upper organic layer was separated and evaporated to dryness at 30°C in a nitrogen evaporator under a gentle stream of nitrogen. The residue was reconstituted with 100 µl mobile phase (buffer: methanol in the ratio 1:1), centrifuged and the supernatant was subjected to RP-HPLC analysis. Injection volume was 50 µl.

Pharmacokinetic and statistical analysis

All pharmacokinetics parameters were determined by non-compartmental analysis using Winnonlin software®. Plasma concentration versus time curves was constructed.

Comparisons between the control and the treated group were performed by analysis of variance followed by one way ANOVA by Dunnett's test using Graph Pad Prism®.

Table 1: Percent w/w content of principal phytoconstituents of *W. somnifera* (withaferin-A and withanolide-A) in ethanolic, methanolic, hydromethanolic and aqueous extracts

Crude extracts	Percent (w/w) content	
	Withaferin - A	Withanolide-A
Ethanolic	0.09 ± 0.001	0.15 ± 0.007
Methanolic	0.16 ± 0.009	0.24 ± 0.025
Hydromethanolic	0.065 ± 0.001	0.04 ± 0.005
Aqueous	0.05 ± 0.002	0.01 ± 0.001

Data are expressed Mean ± standard deviation (SD, n=3)

Table 2: Pharmacokinetic parameters of Phenacetin after administration in rats in the presence and absence of oral administration of methanolic extract of *Withania somnifera*

	Vehicle treated	Treated with methanol extract of WS
Ke (hr ⁻¹)	0.169	0.329*
T _{1/2} (hr)	4.13	2.370*
C _{max} (µg/mL)	77.178	71.003
T _{max} (h)	0.5	0.500
AUC(0-24h)	560.861	386.547*

T_{max}, time to reach the peak plasma concentration; C_{max}, peak plasma concentration; T_{1/2}, apparent elimination half-time; Ke, elimination rate constant; AUC, area under the plasma concentration–time curve, Values are expressed as Mean ± SEM; n=6 in each group, *p<0.05 when Test Group (Group II) compared with Control (Group I) using One way ANOVA by Dunnett's test.

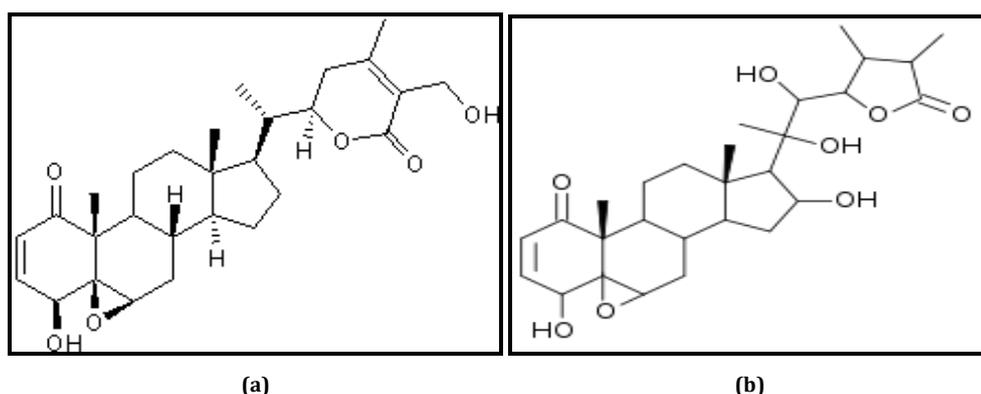


Fig. 1: Structure of principal phytoconstituents of *Withania somnifera*, withaferin-A (a) and withanolide-A (b)

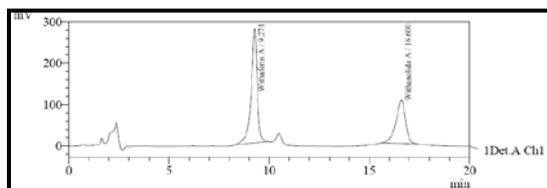


Fig. 2: HPLC Chromatogram of principal phytoconstituents of *Withania somnifera* (Withaferin-A & Withanolide-A)

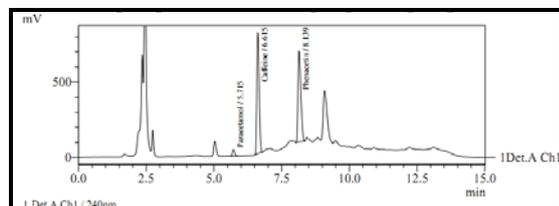


Fig. 3: Representative chromatogram of paracetamol, caffeine and phenacetin in rat liver microsomes

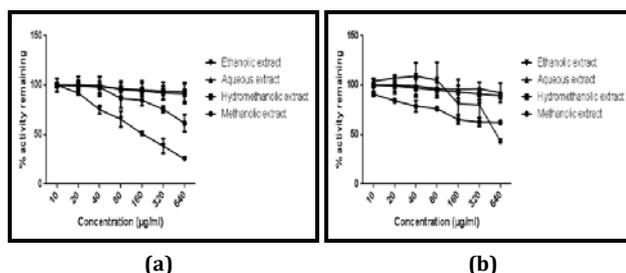


Fig. 4: Percent activity remaining in presence of increasing concentrations of crude extracts of *Withania somnifera* in RLM (a) and HLM (b). Values are expressed as Mean \pm SD (n=3)

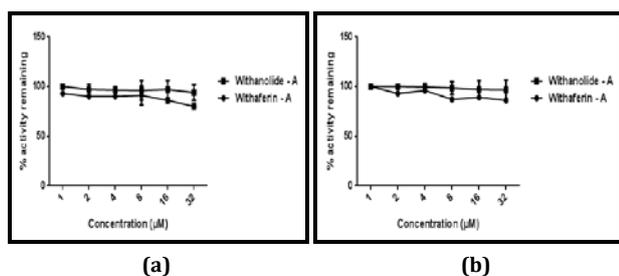


Fig. 5: Percent activity remaining in presence of increasing concentration of principal phytoconstituents (withaferin-A and withanolide-A) in RLM (a) and HLM (b). Values are expressed as Mean \pm SD (n=3)

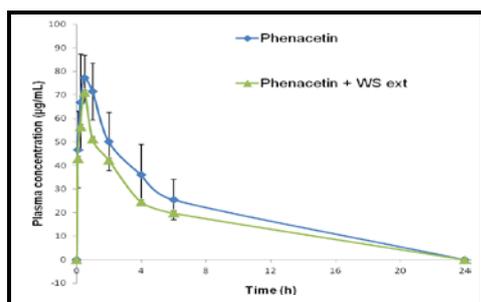


Fig. 6: Plasma concentration - time curve of Phenacetin; 200mg/kg in rats after oral administration. Phenacetin was administered to animals after oral treatment of methanolic extract of WS at a dose 500mg/kg (test group) and treated with vehicle (control group) for 10 days. Values for each time point expressed as Mean \pm SD (n=6)

RESULTS

Quantification of principal phytoconstituents in crude extracts of WS using RP-HPLC

The quantitative analysis was performed by RP-HPLC under the isocratic conditions. The separation was performed with the mobile phase consisting of acetonitrile and water in the ratio of 40:60 (v/v). Linearity of WA & WL-A was tested by linear regression analysis and found to be linear in the concentration range 10–320 μ g/ml with a correlation coefficient (r^2) of 0.9895 and 0.9999 respectively. The percentage content of WA & WL-A present in the crude extracts of WS is as represented in table 1. Fig. 2 shows representative chromatogram of WA & WL-A standards.

In vitro CYP1A inhibition assay

Crude extracts of *Withania somnifera* (10 – 640 μ g/ml) and phytoconstituents (1 – 32 μ M) were evaluated for the CYP1A inhibitory activity in both rat and human liver microsomes (n=3). Fig. 3 shows a representative chromatogram of paracetamol, phenacetin and caffeine (IS) spiked in microsomes. The average percentage activity remaining after incubating at different concentrations of crude extracts of WS, WA & WL-A, individually, in RLM and HLM is graphically represented in fig. 4 & 5. IC_{50} values of all the crude extracts of *Withania somnifera*, WA and WL-A were found to be >500 μ g/ml and >32 μ M, respectively for CYP1A, both in rats and humans. IC_{50} values <100 μ g/ml for herbal extracts and <10 μ M for phytoconstituents are considered to be potent inhibitors of CYP enzymes [21]. Results indicated that *Withania somnifera* and its principal phytoconstituents (WA & WL-A) showed no significant inhibition of CYP1A enzyme in rats and humans.

In vivo pharmacokinetic study

In vivo study in rats was performed to evaluate the induction potential of clinically relevant methanolic extract of *Withania Somnifera* [20] for CYP1A enzyme in rats. This was achieved by comparing the pharmacokinetic parameters [Area under Curve (AUC), C_{max} , T_{max} , apparent elimination rate constant (Ke) and half-life ($T_{1/2}$)] of CYP1A specific probe substrate, phenacetin, at a dose of 200mg/kg, p. o. [22] when administered alone and after oral administration of methanolic extract of *Withania somnifera* at dose of 500mg/kg, p. o. [20] for 10days. Pharmacokinetics parameters for each group were calculated by Winnonlin software is summarized in table 2. Maximum plasma concentration (C_{max}) obtained in group I and II was not significantly different. The elimination half-life ($t_{1/2}$) of group II (2.36 \pm 0.5h) and as compared to group I (4.13 \pm 0.61h) is indicative of fast elimination of drug. It was further supported by elimination rate constant for group II as 0.33 \pm 0.05h⁻¹ in comparison with group I which is 0.17 \pm 0.02 h⁻¹. Group II showed lower AUC value of 386.54 \pm 16.41 μ g-h/ml in comparison with Group I whose AUC was 560.86 \pm 55.8 μ g-h/ml. Plasma concentration time profile of phenacetin in rats treated and untreated with methanolic extract of WS is shown in fig. 6.

Animal group treated with methanolic extract of *Withania somnifera* showed approximately 2 fold increase in elimination rate constant and approximately 30% decrease in AUC of phenacetin as compared to the group treated with phenacetin alone. Hence the pharmacokinetic study indicates faster elimination of phenacetin from rat body after administration of methanolic extract of *Withania somnifera* which could be due to induction of CYP1A in male Wistar rats.

DISCUSSION

Withania somnifera (WS) is an important herb in the Ayurvedic and indigenous medical systems for over 3000 years [23]. The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds, including ergostane tpesteroidal lactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidenone, withasomniferols A-C, withanone etc. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X) [24,25]. Steroidal lactones present in WS are found to bind

and inhibit vimentin. They are also found to be a potent inhibitor of angiogenesis and inhibit both NF- κ B and Sp1 Transcription factor activity. It also down regulates VEGF gene expression. The plant has been widely studied for their various pharmacological activities like antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, anti-inflammatory, antitumor properties. Various other effects like immunomodulation, hypolipidemic, antibacterial, cardiovascular protection, sexual behavior, tolerance and dependence have also been studied [20].

The use of herbs as alternative and complementary therapy has increased worldwide. Patients taking herbs together with prescribed western medication are at a potential risk of herb–drug interactions. Some patients self-medicate with several different herbs and herbal preparations without their doctor's recommendation [26]. *Withania somnifera* is widely used as an alternative to western treatments. However, until now there has been no requirement to evaluate the potential for drug interactions, adverse effects, toxicity or even death from using dietary supplements by dietary supplement manufacturers. Therefore, the risk of herb–drug interactions is increasing.

Cytochrome P₄₅₀ (CYP)1 family members, CYP1A1, CYP1A2 and CYP1B1 which are under the transcriptional regulation of the AhR receptor, are known for their induction by and catalysis of the ubiquitous poly aromatic hydrocarbons (PAHs) present in cigarette smoke, industrial dyes and agricultural pesticides. Expressed in different amounts in the liver (CYP1A2) and extra-hepatic organs (CYP1A1 and CYP1B1) these enzymes in particular CYP1A1 and CYP1B1 catalyze critical conversions to form the ultimate carcinogen [27].

Phenacetin is a recommended probe substrate for CYP1A for *in vitro* & *in vivo* interaction studies. Phenacetin has been found to be almost exclusively metabolized by CYP1A2 to its metabolite acetaminophen.

CYP1A1 activates polycyclic aromatic hydrocarbons to biologically reactive metabolites that interact with DNA resulting in chemical carcinogenesis. CYP1A1 is induced by a large group of environmental chemicals including the polycyclic aromatic hydrocarbons such as 3-methyl cholanthrene, β -naphtho-flavone, benzo [a] pyrene, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) amongst others. An understanding of CYP1A regulation is important for determining and assessing chemical carcinogenesis. Induction of CYP1A has been shown to be mediated through the activation of the aryl hydrocarbon receptor (AhR) [28].

The aim of the present study was to investigate the interaction potential of crude extracts of WS, WA & WL–A, the principal phytoconstituents, with CYP1A enzyme *in vitro* in rat and human liver microsomes and *in vivo* in male Wistar rats.

In vitro investigation in rat and human liver microsomes indicated that the crude extracts of WS, WA and WL–A might not lead to substantial drug herb interactions, since their IC₅₀ values were greater than 640 μ g/ml and 32 μ M, respectively in both rats and humans. The results of this study were in accordance with the results put forth in our previous study of interaction of *W. somnifera* with CYP3A enzyme both in rat & human liver microsomes. The study showed no *in vitro* & *in vivo* inhibition with respect to CYP3A enzyme, thus indicating that *W. somnifera* may not have any potential to lead any serious herb- drug interactions involved with CYP3A substrates both in rats and humans [29].

In vivo pharmacokinetic study of co-administration of methanol extract of WS and phenacetin, revealed that the methanolic extract lead to an approximately 1.5 fold (31 %) decrease in AUC_{0-24h} ($p < 0.05$). Elimination rate constant (ke) increased 2 fold (48 %) and half-life (t_{1/2}) decreased 1.8 fold (43 %). Decrease in AUC_{0-24h} and increased Ke of phenacetin in rats treated with the extract indicates that the extract of WS could have led to an induction of CYP1A enzyme in rats. No significant differences were observed in maximum plasma concentration (C_{max}) in the test and control groups. Herbal extract of WS showed moderate, but significant induction of CYP1A enzyme in rats. This could lead to decrease in therapeutic efficiency of co-administered drugs that are CYP1A substrates with narrow therapeutic index eg. theophylline [30].

CONCLUSION

The findings from this study suggested that the crude extracts of *Withania somnifera* and its principle phytoconstituents, (WA & WL–A) showed no *in vitro* CYP1A inhibition in rats and humans. *In vivo* evaluation of methanolic extract of *Withania somnifera* showed that it leads to induction of CYP1A enzyme in rats which could produce untoward herb-drug interactions. Certain major factors of metabolism such as competition between co-administered drugs, unspecific interactions with proteins and enzyme induction due to chronic intake are not addressed in this study. However, data presented in this study clearly suggested that *Withania somnifera* may have the potential to induce the metabolism of certain co-administered drugs which are specific substrate of CYP1A enzyme and thus may lead to failure of specific pharmacotherapy.

ABBREVIATION

CYP, Cytochrome P450; WS, *Withania somnifera*; WA, Withaferin-A; WL-A, Withanolide-A, DMSO, dimethyl sulfoxide; HDI, herb drug interactions; HLM, human liver Microsomes; NADPH, Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Alice Varghese for providing the valuable insights for carrying out the potential research work in our laboratory. The authors would like to thank Department of Biotechnology, New Delhi, India for providing financial support through project grant [DBT Project no: BT/PR14460/PBD/17/703/2010] for the present research work to Shobhaben Pratapbhai Patel, School of Pharmacy and Technology Management, SVKM's NMIMS. The authors would like to thank Dr. R. S. Gaud; Dean of SPP-SPTM, NMIMS, Mumbai for providing support and necessary facilities. The authors would also like to thank Mr. Laxman Sawant; Senior Research Officer, Natural Remedies, Bangalore, India for providing gift sample of withaferin–A & withanolide-A.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

REFERENCES

1. Ponnusankar S, Pandit S, Babu R, Bandyopadhyay A, Mukherjee PK. Cytochrome P450 inhibitory potential of Triphala—A rasayana from ayurveda. *J Ethnopharmacol* 2011;133:120–5.
2. Obach SR. Inhibition of human cytochrome P450 enzymes by constituents of St. John's wort, an herbal preparation used in the treatment of depression. *J Pharmacol Exp Ther* 2010;294:88-95.
3. Liwei Hu, Wen Xu, Xi Zhang, Juan Su, Xinru Liu, Haiyun Li, et al. *In vitro* and *in vivo* evaluations of Cytochrome P450 1A2 interactions with nuciferine. *J Pharm Pharmacol* 2010;62:658–62.
4. Pius SF, Bouic PJ, Rosenkranz B. An overview of the evidence and mechanisms of herb–drug interactions. *Front Pharmacol* 2012;3:1–19.
5. Guengerich PF. Cytochrome P450 Enzymes. *Am Sci* 1993;81(5):440-7.
6. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern Med Rev* 2000;5:334-46.
7. Bhatnagar M, Sisodia SS, Bhatnagar R. Antiulcer and antioxidant activity of *Asparagus racemosus* WILLD and *Withania somnifera* DUNAL in rats. *Ann NY Acad Sci* 2005;1056:261-78.
8. Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic foot shock stress induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J Ethnopharmacol* 2001;74:1-6.
9. Gupta SK, Dua A, Vohra BP. *Withania somnifera* (ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications. *Drug Metabol Drug Interact* 2003;19:211-22.
10. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J Ethnopharmacol* 2007;71:23-43.

11. Cooley K, Szczurko O, Perri D, Mills EJ, Bernhardt B, Zhou Q, et al. Naturopathic care for anxiety: a randomized controlled trial ISRCTN78958974. PLoS One 2009;4:6628.
12. Ahmad MK, Mahdi AA, Shukla KK, Islam N, Rajender S, Madhukar D, et al. Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. Fertil Steril 2010;94:989-96.
13. Murthy V, Ranjekar MR, Ramassamy PK, Deshpande C. Scientific basis for the use of Indian ayurvedic medicinal plants in the treatment of neurodegenerative disorders: ashwagandha. Cent Nerv Syst Agents Med Chem 2010;10:238-46.
14. Chaurasia ND, Uniyal GC, Lal P. Analysis of withanolides in root and leaf of *withania somnifera* by HPLC with photodiode array and evaporative light scattering detection. Phytochem Anal 2008;19:148-54.
15. Pradnya S, Walawalkar PS Serai, Iyer KR. Isolation and catalytic competence of different animal liver microsomal fractions prepared by calcium – aggregation method. Indian J Pharm Sci 2006;68(2):262-5.
16. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
17. Wrighton S, Ring B, Vandenbranden M. The use of *in vitro* metabolism techniques in the planning and interpretation of drug safety studies. Toxicol Pathol 1995;23(2):199-208.
18. Varghese A, Savai J, Pandita N, Gaud R. Species related differences in *in vitro* metabolism of phenacetin, a probe substrate for CYP1A enzyme, and enzyme kinetic parameters of phenacetin. Int J Pharm Med Bio Sci 2014;3(2):21-30.
19. Guidance for Industry. Drug Interactions Studies: study design, data analysis and implications for dosing and labeling; 2006. /http://www.fda.gov/cder, http://www.fda.gov/cder/guidance/6695dft.pdf. Accessed 26 October 2014.
20. Girdhari LG, Rana AC. Plant review withania somnifera (Ashwagandha): a review. Pharmacogn Rev 2007;1(1):129-36.
21. Yan P. *In vitro* modulatory effects of *Andrographis paniculata*, *Centella asiatica* and *Orthosiphon stamineus* on cytochrome P450 2C19 (CYP2C19). J Ethnopharmacol 2011;133:881-7.
22. Mirosław M, Katarzyna ZS, Marta J, Monika M. *In vivo* effect of diallyl sulfide and cimetidine on phenacetin metabolism and bioavailability in rat. Acta Bio Polonica 2002;49(1):249-56.
23. Weiner MA, Weiner J. Ashwagandha (India ginseng). In: Herbs that Heal. Quantum Books, Mill Valley (CA); 1994.
24. Elsakka M, Grigorescu E, Stanescu U, Stanescu U, Dorneanu V. New data referring to chemistry of *Withania somnifera* species. Rev Med Chir Soc Med Nat Iasi 1990;94(2):385-7.
25. Ganzera M, Choudhary MI, Khan IA. Quantitative HPLC analysis of withanolides in *Withania somnifera*. Fitoterapia 2003;74(1-2):68-76.
26. Kelly JP. Recent trends in use of herbal and other natural products. Arch Intern Med 2005;165:281-6.
27. Badala S. Cytochrome P450 1 enzyme inhibition and anticancer potential of chromene amides from amyris plumier. Fitoterapia 2011;82:230-6.
28. Gonzalez FJ, Yu AM. Cytochrome P450 and xenobiotic receptor humanized mice. Ann Rev Pharmacol Toxicol 2006;46:41-64.
29. Savai J, Varghese A, Pandita N. Lack of the cytochrome P450 3A interaction of methanolic extract of *Withania somnifera*, Withaferin A, Withanolide A and Withanoside IV. J Pharm Negat Results 2013;4(1):26-32.
30. Levy G, Koysooko R. Pharmacokinetic analysis of the effect of theophylline on pulmonary function in asthmatic children. J Pediatr 1975;86:789-93.