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Original Article

Effect of Aqueous Root Extract of *Decalepis hamiltonii* on Lopinavir Pharmacokinetics and Midazolam Pharmacodynamics

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ABSTRACT

Background: The traditional plant *Decalepis hamiltonii*, indigenous to southern India, is a key component in the well-known herbal beverage nannari. It has also been utilised as a general vitalizer and paediatric rejuvenator in siddha, ayurveda, and folk medicine. However, it has been demonstrated that this plant's phytoconstituents can modulate CYP450 enzymes, which are crucial for determining the fate of xenobiotics within the body. It has not yet been observed that this plant's modulatory property would result in herb-drug interactions when used in conjunction with xenobiotics. **Materials and Methods:** The goal of the current investigation was to determine how *Decalepis hamiltonii* affected the pharmacokinetics of the CYP3A substrate lopinavir and the pharmacodynamics of another CYP3A substrate midazolam, in rats. An *in vivo* pharmacokinetic study of oral lopinavir (35.50 mg/kg), and the effects of Aqueous Root Extract of *Decalepis hamiltonii* (AREDH) pretreatment on the pharmacokinetic parameters were evaluated. Further the acute and chronic pretreatment of AREDH on pharmacodynamics of midazolam was assessed through measuring hypnotic responses produced in rats. **Results:** The findings showed that, in comparison to the control, AREDH pretreatment significantly decreased the area under the concentration-time curve (AUC), while a small but not statistically significant change was seen in the half-life of lopinavir (T1/2 k10), the amount of time needed to reach the peak plasma concentration (T_{max}), and the elimination rate constant (k10). In addition, AREDH pretreated groups had significantly less sleep duration than the control group irrespective of the pretreatment duration. However, there was no discernible difference in sleep latency. **Conclusion:** Conclusively, it suggests that *Decalepis hamiltonii* caused CYP3A-mediated increased metabolism via inducing the enzyme, which significantly decreased the oral bioavailability of lopinavir. Clinical investigations are necessary to assess this interaction's therapeutic importance in more detail.

Keywords: *Decalepis hamiltonii*, Herb-drug interactions, Lopinavir, Midazolam, CYP3A, Pharmacokinetics, Pharmacodynamics.

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INTRODUCTION

Throughout history, medicinal herbs have played a crucial role in providing treatment for various ailments, making significant contributions to healthcare. Ancient civilizations placed great emphasis on understanding the healing properties of medicinal plants. Even today, plants continue to hold importance in medicine.[1] Their low cost, efficacy, and cultural significance have led nearly 80% of people in low- or middle-income countries to rely on traditional plant-based medicine for their healthcare needs.[2,3]

However, when herbs and drugs are consumed simultaneously, there is a potential for clinically significant interactions. Many phytochemicals naturally present in herbs can act as substrates for transporters or enzymes that affect the processing of pharmaceuticals in the body. This can impede the absorption, distribution, metabolism, and elimination of drugs. Conversely, the phytochemicals in medicinal plants can also interact with nuclear receptors responsible for the expression of transporters or enzymes that play a crucial role in determining the fate of drugs.[4-6] These interactions can have implications such as altered drug concentrations, pathogen resistance, toxicity, or treatment failure. Currently, a significant portion of the global population relies on herbal medications for treating various ailments.[7] As

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more drugs or plant-based medications are used in combination, the likelihood of herb-drug interactions increases significantly. However, our understanding of these interactions is limited, as most herbs have not been extensively studied in terms of their specific interactions with drugs. Unlike pharmaceuticals, herbal supplements are often not subject to the same regulations and rigorous clinical testing prior to their availability in the market. Additionally, studies on prescription medications rarely take into account herb-drug interactions.[2]

Decalepis hamiltonii (*D. hamiltonii*) is one such medicinally important herb which is a woody, glabrous, monotypic, climbing shrub of the Asclepiadaceae family. It is in the verge of extinction and prefers to grow along rocky valleys, big rock boulders, in the rocky crevices and small embankments where there is thick vegetation, mostly in moist deciduous forests at a height from 300-1200 m.[8] It is indigenous to Andhra Pradesh, (Chittoor, Nellore, Anantapur and Cuddapah districts), Karnataka (Hassan, Mysore, Bellary, Tumkur and Kolar) and in Tamil Nadu (Chengalpattu, Coimbatore, Dharampuri and Niligiri).[9] Swallow root, Sugandha pala, and Mavilinga kizhngu are the common names for the roots of *D. hamiltonii* in English, Telugu, and Tamil, respectively.[10] In Ayurveda, the plant is referred to as Ananthamula. In Siddha, Ayurveda, and folk medicinal traditions, it has been utilised in paediatric rejuvenation and as a general vitaliser.[11] The roots have high antioxidant activity and are utilised in the preparation of pickles.[12,13] Traditionally, the roots have been used for blood purification as well as the treatment of intrinsic haemorrhage, fever Kushtha, wound healing, bronchial asthma, food poisoning and erysipelas. Further, different tribes around India have used roots of *D. hamiltonii* for several purposes.[14,15] The 'Kani' tribes native to western ghats use it as an effective remedy for peptic ulcer and as a rejuvenating tonic, appetizer, blood purifier and preservative. Moreover, roots have also been known to possess the anti-microbial activity against foodborne pathogens.[16] In southern India and other Asian nations like Indonesia and Sri Lanka, the roots are used as an edible food and in a locally produced health beverage called Nannari sharbath that is tend to reduce body heat.[17] In the summer, the beverage is used to satisfy thirst and functions as a hepatoprotective agent, which is beneficial to stomach health.[18] Besides in food industry roots also have an application as substitute for vanillin and are also pickled.[10,13] Numerous pharmacological effects of *D. hamiltonii* have been reported through both *in vitro* and *in vivo* models, including anti-inflammatory activity,[19] neuroprotective activity, hepatoprotective activity, cytoprotective and antioxidant activity.[20] and antimicrobial activity.[17] Further, a rich milieu of phytochemicals has been identified and isolated from the plant *D. hamiltonii*. Phenolic compounds were found to be abundant in *D. hamiltonii* tuberous roots, predominantly 2-hydroxy-4-methoxy benzaldehyde (HMB), which possess abundant medicinal properties.[21,22] It is a volatile substance which is an isomer of vanillin, the main flavouring compound found in the roots of *D. hamiltonii*. Also, other phenolic acids like α-amyrin, lupeol, β-amyrin, α-amyrin acetate, lupeol acetate, β-amyrin acetate,[22] caffeic acid, cinnamic acid, syringic acid, ferulic acid, protocatechuic acid, p-coumaric acid, gallic acid, gentisic acid etc., were isolated from aqueous and methanolic root extracts.[23] Furthermore, the volatile oils were also reported in *D. hamiltonii* tuberous roots include salicylaldehyde, benzaldehyde, methyl salicylate, benzyl alcohol, 2-phenyl ethyl alcohol, ethyl salicylate, p-anisaldehyde and vanillin.[11] Though it hasn't been proven that *D. hamiltonii* is toxic towards humans, it is evidenced from various studies that micromolar concentrations of phenolic chemical constituents like 2-Hydroxy Methyl Benzoic acid and Vanillin exhibited toxic effect towards Aplocheilus Panchax, a fresh water fish.

Moreover, research suggests that most of these phenolic acids isolated from the plant resources have the potential of interfering with the pharmacokinetics and pharmacodynamics (PK/PD) of the several drugs resulting in herb-drug interactions via modulating several enzymes and proteins like CYP450 enzymes, Organic Anion Transporters (OAT) and P-glycoprotein (P-gp) function.[24-26] Among which CYP450 enzymes are significant determinants of a drug's PK/PD. Over 60% of currently used therapeutic drugs are bio transformed by CYP3A enzymes, one of the predominant P450 enzymes in liver and extra-hepatic organs like gut. CYP3A enzymes play a significant role in the oxidation of xenobiotics. In human liver microsomes, one of the most common P450 isoforms for drug metabolism is CYP3A4, which accounts for around 40% of all P450s. In the adult liver and small intestine, CYP3A4 is the most prevalent P450 enzyme, whereas CYP3A5 is expressed polymorphically.[27-29] On the contrary, phenolic acids isolated from *D. hamiltonii* like caffeic acid, vanillin, syringic acid, ferulic acid, gallic acid etc, are demonstrated as potential modulators of CYP450 enzymes,[30-33] OAT[26,34] and P-gp function,[24,35-37] by various studies. Further, *D. hamiltonii* is also reported to possess modulating property towards cytochrome P450 enzymes,[10] which results in substantial herb-drug interactions when ingested along with other drugs. Additionally, our previous *in silico* study revealed that phytochemicals of *D. hamiltonii* are being metabolized by CYP450 enzymes like CYP3A4, CYP2D6, and CYP2C9.[10] Moreover, the binding affinity of the phytochemicals of *D. hamiltonii* towards CYP enzymes was significant specifically with CYP3A4. This results in the competition for the binding site when co-administered with other drugs precipitating herb-drug interactions. *D. hamiltonii* being an important ingredient of the most famous and frequently consumed herbal drink of peninsular India, it interested us in opting it to elucidate its possible herb-drug interactions. Hence, in the present research, we aimed to assess the CYP3A modulatory potential of Aqueous Root Extract of *D. hamiltonii* (AREDH) through assessing pharmacokinetics of Lopinavir and pharmacodynamics of midazolam through measuring the sleep latency produced in rats. Lopinavir and midazolam being

very well-known substrates of CYP3A, were utilized as probe substrates in the present study.

MATERIALS AND MATERIALS

Midazolam (MEZOLAM-10 mL; 1 mg/mL), Ketoconazole (NIZRAL; 200 mg), Rifampicin (R-CIN 450; 450 mg), were procured from local pharmacy and Lopinavir APA obtained as a gift sample from Hetero labs, Hyderabad. HPLC grade Acetonitrile, Methanol, water, Ethanol, Methanol, Orthophosphoric acid, Potassium di-hydrogen phosphate, Carboxy Methyl Cellulose (CMC) was obtained from Merck, India.

Collection of plant material

The roots of *Decalepis hamiltonii* were acquired from a vendor in Kurnool town and were collected from Nallamalla forest, Nandyal region, in the month of December and certified by Dr. K. Madhava Chetty, Plant Taxonomist, SV University, Tirupati, with voucher number 0549.

Preparation of plant extract

The obtained roots were ground into a coarse powder, mixed with nine parts de-mineralized water at 50°C, steeped overnight and filtered the next day. The resulting filtrate was recognised as aqueous extract of root of *Decalepis hamiltonii*. Weight per millilitre was calculated using loss on drying.

Animals

The Wistar strain male albino rats weighing between 150 and 200 g were obtained from Raghavendra Enterprises Pvt. Ltd. in Bangalore, India. All the animals were housed to acclimatize to laboratory conditions for one week at ambient temperature (22±1°C), relative humidity (55±5%), 12 hr/12 hr light-dark cycle, and provided with a healthy pellet diet and water ad libitum. Prior to the start of the experiment, the rats underwent a one-week acclimatization period in the laboratory to adapt to the new environment. All experimental procedures were conducted in accordance with the guidelines and regulations set by the Institutional Animals Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy (approval number: SPSP/1016/PO/Re/S/06/CPCSEA/2022/02).

Experimental Design

The animals were divided into four groups for each experiment:

1. Normal group (Control): Rats in this group received no pretreatment and were administered midazolam.
2. Inducer group: Rats in this group were pretreated with the inducer (Rifampicin) for either 2 days (acute) or 14 days (chronic) and then administered midazolam.
3. Test group: Rats in this group were pretreated with AREDH for either 2 days (acute) or 14 days (chronic) and then administered midazolam.
4. Inhibitor group: Rats in this group were pretreated with the inhibitor (ketoconazole) and then administered midazolam.

Group I (Test Group): Rats pretreated with AREDH for 14 days and administered lopinavir on the 14th day. Group II (Control Group): Rats administered lopinavir on the 14th day without AREDH pretreatment. The test group received both AREDH for 14 days and lopinavir on 14th day, whereas the control group only received lopinavir on 14th day. The data are shown as mean±SD (n=6).

Effect of AREDH treatment on pharmacokinetics of Lopinavir

The obtained rats were randomized into two groups with six animals in each. Group I: received normal saline for 14 days and group II: received AREDH (200 mg/kg p.o.) for 14 days. The animal dose of the drugs was calculated using the formula:

$$\text{Animal dose} = \text{human dose/adult body weight} \times 6.2^{38}$$

After the pre-treatment is finished on the 14th day, Lopinavir (35.50 mg/kg p.o.) was administered to the overnight fasted rats of both groups. It was followed by collection of blood samples through retro-orbital puncturing at the following sampling timepoints, 1, 1.5, 2, 2.5, 3.5, 5.5, and 9 hr. Plasma was isolated from blood after 30 min of blood collection by centrifugation at 5000 rpm for 15 min, and it was then stored at -20°C for 12 hr. The stored plasma samples were thawed to room temperature the next morning, and 50 µL of the sample was collected. To which four parts of acetonitrile was then added, and the mixture was vortexed for 15 min. Later the samples were centrifuged at 4°C for 20 min at 7000 rpm. The obtained supernatants were transferred to 96-well plates and were reconstituted using mobile phase prior to 15 min of spiking into HPLC. From the standard curve of lopinavir drawn by using the same procedure, the drug concentration in each plasma sample was determined. Each plasma sample's concentration was measured in ng/mL.

Effect of AREDH treatment on sleeping time after the midazolam administration

The obtained rats were randomized into four groups (n=6) normal control, inducer group, test drug group and inhibitor group. The acute and chronic effect of AREDH on sleep latency and sleeping time induced by midazolam in rats was assessed with a washout period of 20 days between the assessments. Normal control rats were given with normal saline; the inducer group rats received Rifampicin 40 mg/kg p.o as pre-treatment; the test drug group rats, received AREDH 200 mg/kg p.o. as pre-treatment and the inhibitor group rats, received Ketoconazole (18 mg/kg

p.o.) as pre-treatment. The pre-treatment was administered for 2 days in the case of acute assessment and 14 days in the case of chronic assessment. An hour after the last pre-treatment dosing, midazolam (15 mg/kg i.p) was administered to all four groups of acute and chronic assessment and the indices of hypnotic effect like sleep latency and sleeping time were recorded. The experiments were carried out in a quiet room where the room temperature was maintained at 22±2ºC and the observers recording the indices of hypnotic effects were blind to the drug treatment. Sleep latency was measured as the interval between midazolam administration and the beginning of losing the righting reflex. The sleeping time was defined as the period between the loss and recovery of the righting reflex.

HPLC analysis of lopinavir

Shimadzu HPLC system with SPD 20A UV visible detector, Dual Pump System, and RP C18 column (Phenomenex Luna, 250 mm x 4.6 mm ID, particle size 5 m) was used to examine the samples. Initially, prior to develop method for lopinavir its solubility was determined and found to be slightly soluble in water but it was freely soluble in methanol and acetonitrile. The detection wavelength was obtained from Indian pharmacopeia and was found to be 210 nm. The mobile phase is composed of phosphate buffer (0.01 M KH_2PO_4) and acetonitrile in the ratio of 40:60 respectively. Both solutions were degassed by sparging with helium. The injection volume was 20 µL. The mobile phase was delivered at 1.0 mL/min. A 200:245:555 (v/v) blends of acetonitrile, methanol, and buffer sodium acetate was utilised as the mobile phase. The buffer was mixed with the organic phase and then its pH was set to 3.4. At a rate of 1 mL/min, the mobile phase was isocratically pumped.[39]

Statistical analysis

'GraphPad prism' version 9.0 was used to statistically analyse the data. All experimental values were presented as Standard Errors of the Mean (SEM), and one-way ANOVA was used to compare values across groups. By using a two-way ANOVA followed by Dunnett's multiples comparison test, the sleep latency and sleep duration of the 2 day and 14-day pretreatment were compared. *p* value <0.05 is rendered significant.

RESULTS

Effect of AREDH treatment on pharmacokinetics of Lopinavir

The plasma concentration-time plots of Lopinavir (35.5 mg/kg; p.o) in normal control and AREDH pre-treated group is illustrated in Figure 1. While Table 1 summarises the mean values of parameters associated with pharmacokinetics of Lopinavir in both the absence and presence of pre-treatment with AREDH. The oral pharmacokinetics of lopinavir were shown to be substantially changed by AREDH (200 mg/kg p.o.) pre-treatment for 14 days compared to the control group where only lopinavir alone was administered. AREDH pre-treated group exhibited significant decrease in parameters like $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ of Lopinavir when compared to the control group. Furthermore, other parameters like Ka, Vd and t1/2 k10 of lopinavir were decreased and parameters like k10, CL and T_{max} were upregulated in the pre-treatment group compared to the control group though the change was not statistically significant.

Mean data of Pharmacokinetic Parameters (k10=Elimination rate constant, ka=Absorption rate constant, t1/2 k10=Elimination half-life, t1/2 ka=Absorption half-life, CL=Clearance, Vd=Volume of distribution, C_{max}=Maximum Concentration in the Plasma, T_{max}=Time taken to reach Maximum Concentration, $AUC_{0\text{-}\infty}$=Area under the curve from 0 to infinity, $AUC_{0\text{-}t}$=Area under the curve from 0 to time t) of lopinavir and their standard error was calculated, when administered via oral route in Control and Test groups.

Acute effect of AREDH on hypnotic reflexes produced by midazolam

The acute effect of AREDH on hypnotic reflexes produced by midazolam was investigated. Figure 2 presents the results of this study. Sleep latency, measured in min, was similar across all four groups, regardless of the two-day pre-treatment with AREDH. There were no significant differences in sleep latency among the groups. However, significant differences (***p<0.001) were observed in sleeping time induced by midazolam between the normal control group and the three pre-treated groups. The test compound showed a significant decrease (70%) in sleeping time compared to the normal control group, similar to the inducer group (36.5%). In contrast, the inhibitor group exhibited a 40% increase in sleep time compared to the normal control group.

Chronic effect of AREDH on hypnotic reflexes produced by midazolam

The results of effect of chronic AREDH pre-treatments on hypnotic reflexes sleep latency and sleeping time produced by midazolam in rat are represented in Figure 3. The trend observed in both sleep latency and sleeping time is similar to acute pre-treatment groups. The sleep latency was nearly similar in all the four groups irrespective of the pre-treatment that was received for two days. There was no significant difference observed in sleep latency among all the groups. On the contrary, all three pre-treated groups exhibited significant difference (***p<0.001) in sleeping time induced by midazolam when compared to normal control group. The percent of influence on the sleeping time was slightly pronounced in the case of chronic pre-treated groups. The test compound exhibited significant decrease (76%) in the sleeping time when compared to normal control group, trend similar to inducer group (45%). However, inhibitor group showed 54% increase in sleep time compared to normal control group.

Table 1: Effect of AREDH on in pharmacokinetic parameters of lopinavir.

Parameter	Mean values±Standard error (*n*=6) (control)	Mean values±Standard error (*n*=6) (test)
Ka (hr^{-1})	4.470±2.420	0.387±0.009
k10 (hr^{-1})	0.098±0.013	0.364±0.009
t1/2 ka (hr)	0.323±0.079	1.794±0.043
t1/2 k10 (hr)	7.738±0.980	1.906±0.048
Vd (mL/kg)	0.110±0.004	0.057±0.000
CL (mL/kg/min)	0.010±0.001	0.020±0.000
T_{max} (hr)	1.398±0.241	2.668±0.066
C_{max} (µg/mL)	273.098±4.574	231.442±2.822
AUC_{0-t} (μgmL^{-1} min)	1844.362±43.605	1425.396±31.188**
$AUC_{0-\infty}$ (μgmL^{-1} min)	3471.586±327.024	1680.719±52.831***

Significant deviation from the control group (p<0.001*** and p=0.001**).

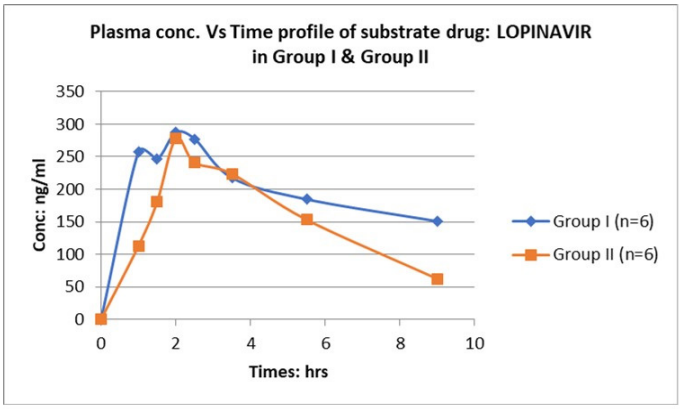

Figure 1: Lopinavir (35.50 mg/kg) plasma drug concentration-time plots in Wistar rats.

DISCUSSION

In a nation like India, the public frequently use a variety of herbal medications on their own or in conjunction with prescription and over-the-counter drugs, assuming naturally derived herbal medications are always safer than synthetically derived drugs. However, these herbs possess rich milieu of chemical constituents that are effective in interacting with the other drugs administered via different mechanisms. Pharmaceuticals and herbal drugs or supplements can interact with one another in two different ways: pharmacokinetically and pharmacodynamically, with pharmacodynamics being significantly dependent on the pharmacokinetics of the drug. The present interest in this study is how pharmacokinetics of the drugs is being affected by these herbal medications or supplements. Pharmacokinetic herb-drug interactions impact the absorption, distribution, metabolism, and excretion of either pharmaceuticals or herbal medicines. These interactions alter the amount of drug required to produce a therapeutic effect by either increasing or reducing the free drug available in the plasma. On the other hand, pharmacodynamic interactions can increase (synergistic or additive activities) or decrease (antagonistic activities) a drug's therapeutic activity.

In the southern regions of India, a refreshing herbal beverage called nannari sharbat is one such supplement that is widely consumed during the months of summer. This popular beverage is prepared from the roots of *Decalepis hamiltonii*, a plant known for its numerous pharmacological properties. Besides in the form of sharbat, the roots are also pickled for consumption. Also, our research group previously conducted an *in silico* study, which revealed significant interactions between the phytoconstituents isolated from aqueous extract of *D. hamiltonii* roots and important enzymes involved in drug metabolism, namely CYP3A4, CYP2D6, and CYP2C9.[10] A total of seven molecules from the extracts when screened through SMARTcyp revealed that they possess minimum of three active sites for all the three CYP enzymes. Additionally, the binding affinity and binding energies were assessed for all seven molecules with CYP3A4, CYP2D6, and CYP2C9, revealing a higher selectivity towards CYP3A4. Moreover, metabolism plays a pivotal role in determining the fate of drugs and herbal medicines in the body, and CYP450 enzymes, particularly CYP3As, play a crucial role in this process. This preliminary research revealed through HPTLC finger printing, that these phytochemicals are being metabolised by CYP3A4, CYP2D6, and CYP2C enzymes.[10] The outcomes suggested that there is significant possibility of competition for metabolism of these phytochemicals with that of drugs which are also substrates for the same enzymes, resulting in herb-drug interactions. The next warranted studies for assessing CYP-inhibition by *Decalepis hamiltonii* were the primary of objective of the present study. The decision to investigate *Decalepis hamiltonii* in our research was also influenced by other key factors. Firstly, the plant has a long-standing history of traditional use in Siddha, Ayurveda, and folk medicine, particularly in the preparation of the renowned nannari beverage. It is also being consumed in the form of pickles. This extensive traditional usage not only highlights the plant's therapeutic properties but also suggests its potential interactions with biological systems, including drug-metabolizing enzymes. Furthermore, previous studies have demonstrated that the phytoconstituents present in *Decalepis hamiltonii* possess the ability to modulate CYP450 enzymes, specifically CYP3A.[30-33] This finding emphasizes the presence of bioactive compounds within the plant that can interact with and influence the activity of these enzymes. Given the crucial role of CYP3A in the metabolism of various drugs, any alteration in its activity can have significant implications for drug metabolism and effectiveness. Investigating the impact of *Decalepis hamiltonii* on CYP3A-mediated metabolism can provide valuable insights into potential interactions with drugs that are substrates for CYP3A, which are commonly prescribed in clinical practice.

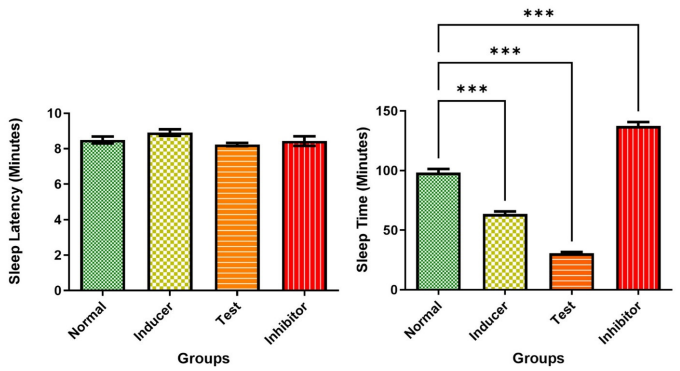

Figure 2: Effect of acute pretreatment of AREDH on hypnotic reflexes of midazolam.

The data of Sleep Latency and Sleep Time in min produced by Midazolam in four groups after treatment with drugs for 2 days is expressed as mean±SEM. Significant difference from the Normal group was designated as ***p<0.001.

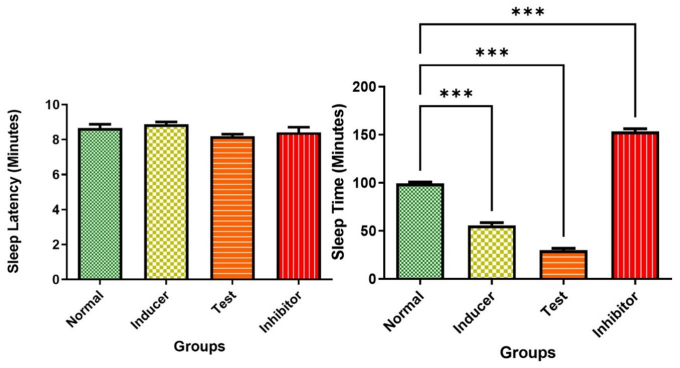

Figure 3: Effect of chronic pretreatment of AREDH on hypnotic reflexes of midazolam.

The data of Sleep Latency and Sleep Time in min produced by Midazolam in four groups after treatment with drugs for 14 days is expressed as mean±SEM. Significant difference from the Normal group was designated as ***p<0.001.

This knowledge is crucial for promoting the safe and effective utilization of both herbal and conventional medicines, particularly when they are used together. Therefore, the selection of *Decalepis hamiltonii* for our investigation is driven by its unique properties, well-established traditional use, and the necessity to evaluate herb-drug interactions, rather than a direct comparison to other dietary plant materials. In the present study, we aimed to investigate the potential herb-drug interactions of AREDH by assessing the pharmacokinetics of lopinavir and the pharmacodynamics of midazolam in rats. Lopinavir, an antiretroviral agent was employed as an appropriate probe drug for the pharmacokinetic studies in the present research. Lopinavir is rapidly metabolised by NADPH and CYP3A4/5 enzyme systems making it poorly bioavailable.[40,41] Due to these characteristics as a CYP3A-specific substrate and its involvement in CYP3A-mediated metabolism, it was opted to assess the possible effect of *Decalepis hamiltonii* on CYP3A enzymes. Hence, by studying the pharmacokinetics of lopinavir in rats pretreated with AREDH, we aimed to investigate the potential impact of AREDH on CYP3A-mediated metabolism and the resulting changes in the bioavailability of lopinavir.

The results of our study indicated a substantial change in the pharmacokinetics of lopinavir in group pre-treated with AREDH when compared to Lopinavir alone treated group. The parameters like AUC_{0-t} and $AUC_{0-\infty}$ were significantly reduced in the AREDH pre-treated group compared to the control group, indicating a total decrease in drug exposure in the plasma. As AUC is often quantified to determine the bioavailability of the drug, this also indicates that AREDH pre-treatment resulted in downregulated bioavailability of lopinavir. Additionally, parameters like C_{max}, Ka, t1/2 k10, and Vd of lopinavir were also slightly diminished in the AREDH pre-treated group compared to the control group, while parameters like k10, CL, and T_{max} were slightly augmented in the AREDH pre-treated group. The increased T_{max} and reduced C_{max} along with other absorption parameters also indicate that AREDH pre-treatment also delayed absorption of Lopinavir. Further, augmented CL and diminished t1/2 k10 in AREDH pre-treated group depicts that lopinavir is being rapidly eliminated making it less available in plasma. In addition to that, it was also evident with an increased CL value of lopinavir in AREDH pre-treatment. All these changes conclusively suggests that AREDH via inducing CYP3A enzyme enhances the metabolism and elimination of lopinavir, leading to decreased levels of the drug available in the plasma. Co-administration of *D. hamiltonii* with lopinavir or other drugs which are substrates for CYP3A should be discouraged, as competition for metabolism compromise their bioavailability and effectiveness resulting in herb-drug interactions.

In our study, we treated the rats with AREDH for duration of 14 days, which is a common practice in pre-clinical studies to evaluate the chronic effects of a substance. Moreover, in southern India it is a usual practice of frequent consumption of *D. hamiltonii* roots not only in the form of nannari in the summers, but also as pickles which could be considered as chronic consumption. Although our study design may not directly correlate with the common way of human consumption, it allows us to assess the long-term impact of AREDH on the pharmacokinetics of lopinavir. It is important to note that pre-clinical studies provide essential insights into the potential interactions and effects of substances, and further clinical studies are required to validate these findings in humans.

Furthermore, we investigated the pharmacodynamic effects of AREDH pre-treatment by measuring the hypnotic reflexes of midazolam. As midazolam is considered as one of the 'gold standard' probe substrates for CYP3A and feasibility in assessing its pharmacodynamics it was employed in our study. The impact of both acute and chronic pre-treatments on midazolam-induced hypnotic reflexes showed similar trends, although the effect was slightly more pronounced in the chronic assessment. Pre-treatment with AREDH did not significantly affect sleep latency, but it had a significant influence on sleeping time. AREDH decreased the

sleep time caused by midazolam compared to the control group. This decrease in sleep time was similar to the positive control group, which was used to induce sleep, although AREDH showed even greater reduction. In contrast, the negative control group (inhibitor group) exhibited increased sleeping time unlike the AREDH group. These findings suggest that AREDH induces the activity of the CYP3A enzyme, responsible for midazolam metabolism. This induction effect led to the rapid elimination of free midazolam from the plasma, reducing its availability to induce hypnosis compared to normal control rats. These findings further support the potential herb-drug interaction between AREDH and drugs metabolized by CYP3A.

In light of our findings, it is important for healthcare professionals to inquire about the use of herbal medicines or supplements when prescribing medications to patients. Patients should be educated about potential herb-drug interactions and encouraged to disclose any herbal or natural product use. This information can help healthcare providers make informed decisions and adjust medication regimens to ensure optimal treatment outcomes. The widespread use of *Decalepis hamiltonii* and other herbal medicines in India emphasizes the need for further research and understanding of herb-drug interactions. While traditional herbal remedies are often perceived as safe, it is crucial to recognize that these substances can have potent pharmacological effects and may interact with conventional medications. Integrating traditional and modern medicine requires a comprehensive evaluation of the safety and efficacy of co-administered treatments.

Moreover, it is worth noting that our study focused on the interaction between AREDH and lopinavir, as well as the pharmacodynamics of midazolam. However, the potential for herb-drug interactions extends beyond these specific cases. Different herbal products may interact with various drugs, and the outcomes can vary depending on the specific substances involved. Therefore, further research is needed to explore the interactions of *Decalepis hamiltonii* with a broader range of drugs to provide a comprehensive understanding of its potential effects.

In conclusion, our study provides evidence of the pharmacokinetic and pharmacodynamic interactions between AREDH, derived from *Decalepis hamiltonii*, and lopinavir and midazolam, respectively. The findings highlight the importance of considering herb-drug interactions in the context of herbal medicine or supplement use, particularly in regions where herbal remedies are prevalent. Further research, including clinical studies, is warranted to validate these findings and expand our understanding of herb-drug interactions involving *Decalepis hamiltonii* and other herbal products. By gaining insights into these interactions, we can enhance patient safety, optimize treatment outcomes, and promote the responsible and informed use of both herbal and conventional medicines.

CONCLUSION

In summary, the present study investigated the effect of *Decalepis hamiltonii* (AREDH) root extract on the pharmacokinetics and pharmacodynamics of lopinavir, a CYP3A-specific substrate, in rats. The findings suggest that AREDH has a significant impact on the metabolism of lopinavir through induction of CYP3A enzymes, leading to a decrease in lopinavir bioavailability. This indicates a potential herb-drug interaction when AREDH and lopinavir are co-administered. The pharmacodynamic assessment using midazolam as a CYP3A substrate showed that AREDH pretreatment decreased the sleeping time induced by midazolam, further supporting its role as a CYP3A inducer. Additionally, the similar outcomes in the acute and chronic pretreatment of AREDH in the pharmacodynamic studies could suggest that a single time consumption of nannari might have greater impact on drugs metabolised by CYP3A. These results indicate that AREDH can modulate CYP3A activity, potentially affecting the therapeutic efficacy and toxicity of co-administered drugs. These findings highlight the importance of considering herb-drug interactions, particularly in regions where herbal medicines are commonly used alongside conventional medications. Patients should be advised to disclose their use of herbal products and medications to ensure the safe and effective use of both.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AREDH: Aqueous root extract of *Decalepis hamiltonii*; **LMIC:** Low-or middle-income countries; **AUC:** Area Under Curve; **HMB:** 2-hydroxy-4-methoxy benzaldehyde; **PK/PD:** Pharmacokinetics and pharmacodynamics; **OAT:** Organic anion transporters; **P-gp:** P-glycoprotein; **IAEC:** Institutional Animals Ethical Committee; **SEM:** Standard errors of the mean; **k10:** Elimination rate constant; **Ka:** Absorption rate constant; **t1/2 k10:** Elimination half-life; **t1/2 ka:** Absorption half-life; **CL:** Clearance; **Vd:** Volume of distribution; **C_{max}:** Maximum Concentration in the Plasma; **T_{max}:** Time taken to reach Maximum Concentration;

$AUC_{0-\infty}$: Area under the curve from 0 to infinity; **AUC_{0-t}:** Area under the curve from 0 to time t.

SUMMARY

In this study, we investigated the impact of Decalepis hamiltonii, a traditional plant commonly used in herbal beverages and traditional medicine, on the pharmacokinetics of the CYP3A substrate lopinavir and the pharmacodynamics of midazolam in rats. Our findings showed that pretreatment with the Aqueous Root Extract of Decalepis hamiltonii (AREDH) resulted in a significant decrease in the oral bioavailability of lopinavir. Additionally, rats pretreated with AREDH exhibited shorter sleep duration compared to the control group, regardless of the pretreatment duration. These results suggest that Decalepis hamiltonii may induce CYP3A enzymes, leading to increased metabolism and reduced effectiveness of lopinavir. Further clinical investigations are needed to understand the therapeutic implications of this interaction in more detail.

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