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CLINICAL TRIALS

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Milk thistle and indinavir: a randomized controlled pharmacokinetics study and meta-analysis

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Abstract *Objectives*: To determine whether ingestion of milk thistle affects the pharmacokinetics of indinavir.
Methods: We conducted a three-period, randomized controlled trial with 16 healthy participants. We randomized participants to milk thistle or control. All participants received initial dosing of indinavir, and baseline indinavir levels were obtained ($AUC_{0\text{-}8}$) (phase I). The active group were then given 450 mg milk-thistle extract capsules to be taken t.i.d. from day 2 to day 30. The control group received no plant extract. On day 29 and day 30, indinavir dosing and sampling was repeated in both groups as before (phase II). After a wash-out period of 7 days, indinavir dosing and sampling were repeated as before (phase III).
Results: All participants completed the trial, but two were excluded from analysis due to protocol violation. There were no significant between-group differences. Active group mean $AUC_{0\text{-}8}$ indinavir decreased by 4.4% (90% CI, −27.5% to −26%, $P=0.78$) from phase I to phase II in the active group, and by 17.3% (90% CI, −37.3% to +9%, $P=0.25$) in phase III. Control group mean $AUC_{0\text{-}8}$ decreased by 21.5% (90% CI, −43% to +8%, $P=0.2$) from phase I to phase II and by 38.5% (90% CI, −55.3% to −15.3%, $P=0.01$) of baseline at phase III. To place our findings in context, milk thistle–indinavir trials were identified through systematic searches of the literature. A meta-analysis of three milk thistle–indinavir trials revealed a non-significant pooled mean difference of 1% in $AUC_{0\text{-}8}$ (95% CI, −53% to 55%, $P=0.97$).
Conclusions: Indinavir levels were not reduced significantly in the presence of milk thistle.

Keywords Milk thistle · Indinavir · Randomized controlled trial · Pharmacokinetics · Drug interactions

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Introduction

Prolonged use of natural health products may alter protease inhibitor (PI) concentrations, potentially producing suboptimal drug concentrations leading to PI resistance [1, 2] in some, and in others increasing exposure resulting in drug toxicity. Milk thistle (*Silybum marianum)* is a herbal remedy commonly used by people with human immunodeficiency virus (HIV) for the management of hepatotoxicity related to highly active anti-retroviral drug therapy (HAART) [3]. In

vitro milk thistle has been shown to significantly induce cytochrome P_{450} (CYP) isoform 3A4 [4, 5] . As HIV-1 PIs are substrates of the CYP3A4 isoform, an interaction may have serious clinical implications by reducing plasma drug concentrations.

Two previous clinical trials, utilizing before-and-after designs to determine milk thistle/indinavir interactions concluded that there was no significant interaction [6, 7], although they showed large decreases of indinavir in plasma within some individuals. These studies were limited by not employing a control group. A control group is necessary to determine interactions as other factors, not associated with the plant extract, may affect pharmacokinetics. Therefore, to more accurately evaluate the potential for a drug interaction, we conducted a randomized controlled trial.

Materials and methods

Protocol

We conducted an open-label study in healthy HIV-negative males aged 18–35 years with normal screening physical and laboratory exams. The study was approved by the Sunnybrook and Women's College Hospital ethics review board, and all participants gave written informed consent. Participants were excluded if they had smoked in the previous 6 months, were taking any dietary supplements, did not properly complete the baseline dosing with indinavir (based on self-report and initial blood concentrations), or had a severe reaction to indinavir or a history of malabsorption. Participants were informed to discontinue all known CYP 3A4 inducers including concomitant medications, alcohol, caffeine and juices for 2 months prior to study initiation.

Phase I All participants ($n=16$) received indinavir 800 mg (Merck Frosst, Canada) taken on an empty stomach every 8 h (three oral doses). Participants arrived at the clinic on study morning 2 and were observed taking the final (4th) dose of indinavir in a fasting state with their first blood draw. Participants were randomized on the morning of day 2 using observed coin-toss matching to receive either milk thistle (three capsules per day; Kare and Hope Ltd., Toronto, ON, Canada, each validated independently to contain 456 mg *Silymarins*) for 28 days from day 2 to day 30 ($n=8$) or no herb ($n=8$). Nine blood samples (5 ml) were collected into vacutainer tubes that contained heparin with the 4th indinavir dose (0 h) and at 0.5, 0.75, 1, 2, 3, 4, 6 and 8 h after the final dose of indinavir. Phlebotomists marked the exact times (minutes) that the blood was drawn. Blood was centrifuged for 15 min to separate the plasma, and plasma aliquots were stored in polypropylene tubes and frozen at −80°C. Participants were instructed to take all herbal capsules separately from meals and were provided with diaries and programmed beepers to assist them in maintaining compliance.

Phase II All participants returned to the clinic on the morning of day 29 in a reported fasting state. Timed blood samples were again collected according to the schedule described above for period 1 after the final (4th) dose of indinavir. Participants in the active group took their final dose of milk thistle with the final dose of indinavir. Blood samples were collected as in phase I. Milk thistle was then discontinued in the active group. We obtained complete blood cell counts (CBCs) and liver function tests to establish safety.

Phase III After a washout period of 1 week, indinavir was again dispensed at a dose of 800 mg (taken orally), fasting every 8 h in both groups. To determine whether there was a rebound to baseline, all participants returned to the clinic in a fasting state on the morning of day 36. At this time, participants received their 4th dose of indinavir and resumed blood draws as per phases I and II. On this last study day, CBCs and liver function tests were performed.

During the blood sampling days, participants arrived at the clinic in a fasting state. All participants were provided with adequate fluid intake throughout and received a standardized dinner 4 h after the final doses of indinavir.

Analysis

The high-performance liquid chromatography (HPLC) system consisted of a 515 HPLC pump, a plus autosampler and an ultraviolet (UV) detector. A plasma sample of 1 ml was mixed with 1 ml of 5% ammonium hydroxide and 200 µl of 6 µg/ml clomipramine hydrochloride. After mixing, 6 ml methyltert-butyl ether was added and each sample was vortexed for 1 min. The upper organic layer was removed, placed in a clean test tube and evaporated to dryness at 50°C under a gentle stream of nitrogen. The residue was reconstituted with 200 µl of 0.1 M citric acid and methanol (equal parts) and then the sample was back-extracted with 3-ml *n*-hexane to remove interfering compounds. A total amount of 150 µl of the lower aqueous layer was injected directly into the LC system. The lower limit of quantification (LOQ) of the method for indinavir from plasma is 0.026 µg/ml (26 ng/ml). All samples were analysed in duplicate. The error on duplicate analysis for all concentrations in the study averaged 4.28% [coefficient of variation (CV)] with a maximum value of 10.86 reported in one sample.

Silybum marianum

We determined which *S. marianum* product to use in this study through laboratory analysis and consensus at the University of Ottawa. Of all products available from health food stores in Central Toronto, we evaluated the

consistency and CYP influence of each product [5] . We additionally determined likelihood of use in the target population by determining reported dose on the bottle, number of tablets and cost. The product utilized in this trial contained an average of 456 mg *S. marianum* per tablet (Kare and Hope Inc., Toronto, ON, USA). Each active group participant received a standardized regimen of one 456-mg tablet three times per day away from meals for 4 weeks.

Statistics

The plasma concentration–time data for indinavir were analyzed by means of noncompartmental methods using Pharsight Win, Nonlin Professional Version 4.01 software (Pharsight Corporation, Mountain View, CA, USA).

Observed times were used for analysis. The principle plasma pharmacokinetic parametres were: maximum observed concentration (C_{max}), time to maximum observed concentration (t_{max}), observed post-dose concentration at the end of the 8-h dosing interval (C_8), area under the concentration–time curve from the time of dosing (hour 0) to the end of the 8-h dosing interval ($AUC_{0\text{-}8}$), and elimination half-life within one 8-h dosing interval ($t_{1/2}$).

All parameters except t_{max} were ln-transformed before statistical analysis. Parameters were analyzed using a general linear model (procGLM) estimation method. We conducted analysis of variance (ANOVA) techniques using type-three sum of squares and incorporating subject, period and group as factors to produce least-squares geometric and arithmetic means and standard errors for ln-transformed and untransformed data, respectively. We conducted linear regression and aggregate ANOVA techniques to determine whether the ln-transformed pharmacokinetic parameters in the control group remained constant over the duration of the study. For logarithmically transformed data, we used intra-individual CV calculated as: $100\% \times \left(e^{\text{Mean square residual(MSR)}} - 1\right)^{1/2}$ and inter-individual CV estimated as: $100\% \times \left(e^{(MS-MSR)/3} - 1\right)^{1/2}$. We performed comparisons between and within groups. From pairwise comparisons of the ln-transformed $AUC_{0\text{-}8}$, C_{max}, $t_{1/2}$ and C_8 data, the ratios of the geometric means and the 90% geometric confidence intervals for the ratios were determined. For the untransformed t_{max} data, the absolute difference in means and the ratio of means and their associated 90% confidence intervals were determined where appropriate. The 90% confidence limits around the ratio of geometric means were calculated relative to the control group or baseline values in period 1 using the appropriate ANOVA error term.

The significance level for each comparison was set at $P = 0.05$.

In order to conduct the meta-analysis, we included all three milk thistle/indinavir studies identified through a systematic review [8]. Pooled analysis of mean differences was conducted using a fixed effects model. We tested for heterogeneity using the Zalen test [9] and Higgins I^2 [9]. A priori explanations of heterogeneity included study design, dosing period and milk-thistle extract. Revman 4.2.5 was used for all meta-analytic procedures (Revman, Copyright 2002–2004, Oxford).

Results

Sixteen participants were randomized and completed the study (see Fig. 1). There were no significant differences between the control and milk-thistle group with regard to age or baseline laboratory characteristics (Table 1). No indinavir doses were reported as missed by any patient, but a mean of three (95%CI. 1.26–4.73) milk-thistle capsules per patient were reported as missed. Pharmacokinetic data from two individuals in the

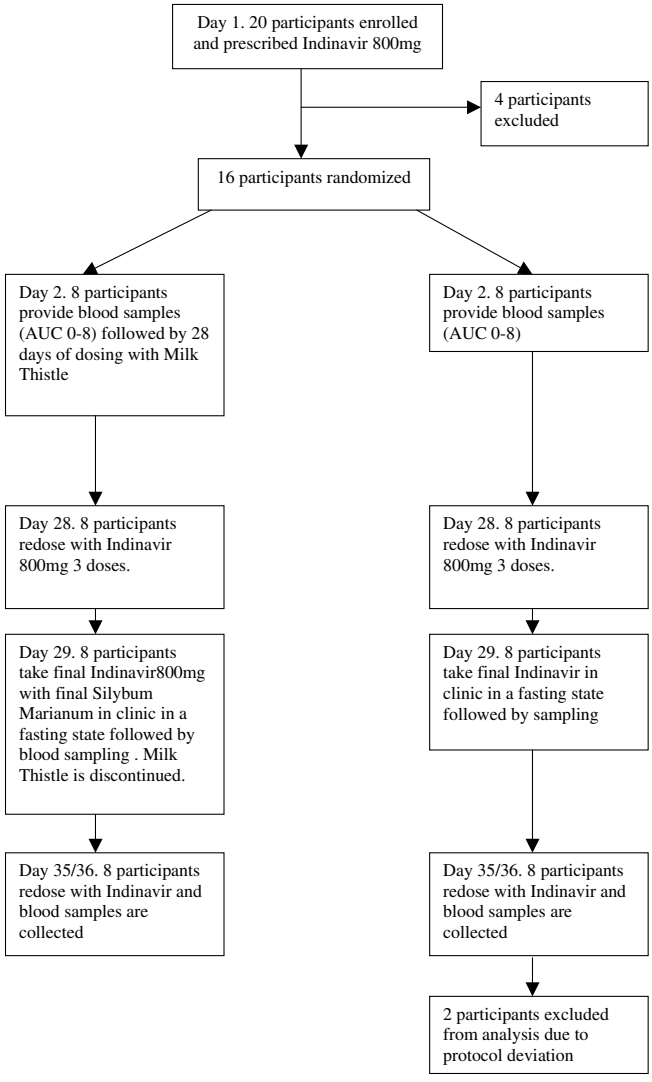

Fig. 1 Flow of participants through trial

Table 1 Characteristics of participants in trial

	Milk thistle	Control
Age (SD), years	25.37 (3.06)	28.25 (3.85)
Body mass index (SD)	24.74 (1.82)	27.71 (5.40)
Hemoglobin (SD), g/dl	153.88 (6.79)	149.63 (6.99)
Leukocytes (SD), $\times 10^9$ cells/l	5.99 (0.75)	6.33 (1.22)
Lactate dehydrogenase (SD), IU/L	140.20 (15.9)	139.88 (30.71)
Aspartate aminotransferase (SD), IU/L	21.00 (6.59)	22.63 (2.07)
Alanine aminotransferase (SD), IU/L	19.50 (12.07)	22.50 (7.52)
Alkaline phosphatase (SD), IU/L	73.88 (11.38)	65.75 (10.12)

There were no significant differences between groups

control group were not evaluable because of high predose concentrations of indinavir of more than 3 μg/ml in one or more periods, indicative of protocol violation. These subjects were excluded from all pharmacostatistical analyses. There were no premature discontinuations. Two patients in the milk-thistle arm reported mild gastrointestinal symptoms, one participant reported paresthesia of the face and dry lips and one participant reported transient vertigo. Four participants in the milk-thistle group and two participants in the control group illustrated increased total bilirubin at the conclusion of the trial (mean total bilirubin elevation 10.3 μmol/l), a known effect of indinavir.

Mean geometric indinavir pharmacokinetic parameters for the active and control groups are summarized in Tables 2 and 3. In both groups, mean indinavir concentrations peaked between 0.7 h and 0.9 h and then decreased with an average elimination half-life of about 1 h.

Comparing pharmacokinetic variables between the active and control groups, there were less than 10% differences in $AUC_{0\text{-}8}$, C_{max} and $t_{1/2}$ values and less than 25% differences in C_8 over all three periods combined (group effect in ANOVA) and in baseline values in period 1 (Table 4). All comparisons were non-significant ($P > 0.31$). Baseline comparison of the treatment groups indicated an $AUC_{0\text{-}8}$ difference of 6.7% (90% CI, $-30.8 + 25.8\%$, $P = 0.6$). Differences in AUC parameters between the control and milk-thistle group and treatment periods are demonstrated in Fig. 2.

There were no significant differences in slopes of AUC or C_{max} versus period plots between the groups. However, after adjusting for baseline differences, we observed a non-significant difference of 21.9% (90% CI, −13.4% to +71.5%, $P = 0.32$) between the experimental group and controls for $AUC_{0\text{-}8}$ in phase II. This difference increased by phase III to 34.3% (90% CI, −4.5% to +89%, $P = 0.14$) but remained non-significant.

The meta-analysis (Fig. 3) demonstrates the different effects observed in the three studies (Table 5) when this plant extract and RCT design is used. The pooled effect remains non-significant (1% decrease in $AUC_{0\text{-}8}$ (95% CI, −53% to −55%, $P = 0.97$). We found no indication of statistical heterogeneity ($P = 0.6$, I^2, 0%).

Discussion

In this trial, milk-thistle ingestion did not significantly alter the pharmacokinetics of indinavir. Previous plant

Table 2 Geometric mean indinavir pharmacokinetic parameters, including paired comparisons

	Geometric mean value			Ratio of means (%) (90% CI)*P* value	
	Phase I	Phase II	Phase III	Phase II to phase I	Phase III to phase I
Active group					
C_{max} (μg/ml)	9.76	9.28	8.14	95.0 (74.3–121.7)0.72	83.4 (65.1–106.7)0.21
$AUC_{0\text{-}8}$ (h μg/ml)	22.7	21.7	18.8	95.6 (72.5–126.0)0.78	82.7 (62.7–109.0)0.25
$t_{1/2}$ (h)	1.1	1.1	1.0	95.2 (85.8–105.8)0.43	92.0 (82.9–102.2)0.18
C_8 (ng/ml)	152	148	125	97.4 (66.5–142.5)0.90	82.6 (56.5–121.0)0.39
Control group					
C_{max} (μg/ml)	9.96	9.03	7.61	90.6 (68.2–120.5)0.56	76.4 (57.5–101.6)0.11
$AUC_{0\text{-}8}$ (h μg/ml)	24.3	19.1	15.0	78.5 (57.0–108.0)0.20	61.5 (44.7–84.7)0.01
$t_{1/2}$ (h)	1.1	1.1	1.3	103.4 (91.6–116.7)0.64	115.9 (102.7–130.8)0.04
C_8 (ng/ml)	181	85.0	105	46.9 (29.6–74.1) < 0.01	57.8 (36.5–91.3)0.05

Table 3

Parameter	Ratio of means, % (90% CI)a P value		Pooled intrasubject CV, %	Pooled intersubject CV, %
	Period 2	Period 3		
C_{max} (μg/mL)	104.9 (75.5-145.6) 0.8011	109.1 (78.6-151.5) 0.6443	29.5	25.4
$AU_{C0\text{-}8}$ (hr·μg/mL)	121.9 (86.6-171.5) 0.3226	134.3 (95.5-189.0) 0.1496	33.2	32.6
$t_{1/2}$ (h)	92.2 (83.6-101.6) 0.1623	79.5 (72.1-87.6) 0.0013	12.3	18.0
C_8 (ng/mL)	208.1 (116.0-373.2) 0.0470	143.1 (79.8-256.6) 0.2898	43.9	58.5

[a]Ratio of geometric mean of active group to geometric mean of control group
[b]Group effect from ANOVA

Table 4 Comparison of geometric mean indinavir pharmacokinetic parameters between active and control groups in period 1 and over all three periods combined

Parameter	Ratio of means (%) (90% CI)[a] *P* value	
	Period 1 (baseline)	Periods 1–3[b]
C_{max} (μg/ml)	98.0 (75.1–127.9)0.8974	102.5 (76.7–136.9)0.8826
AUC_{0-8} (h μg/ml)	93.3 (69.2–125.8)0.6961	110.0 (77.2–156.8)0.6408
$t_{1/2}$ (h)	102.7 (91.7–115.0)0.6952	92.5 (76.8–111.4)0.4688
C_8 (ng/ml)	83.6 (54.2–128.9)0.4834	120.2 (62.8–230.0)0.6231

[a]Ratio of geometric mean of active group to geometric mean of control group
[b]Group effect from ANOVA

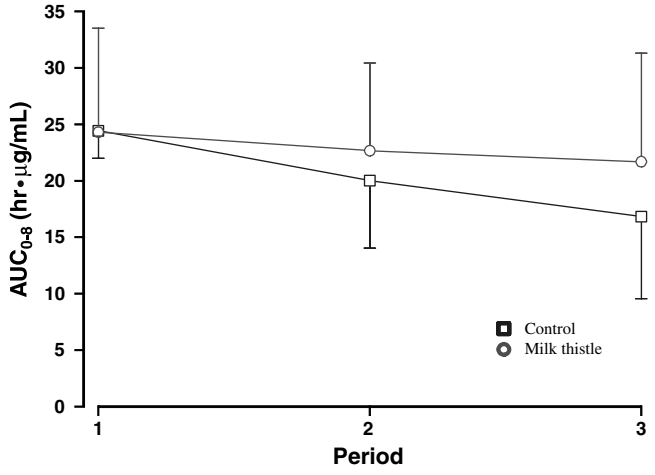

Fig. 2 Indinavir mean (SD) AUC_{0-8} by period for the two groups

extract–PI interaction trials have not utilized control groups and have had variable conclusions determining interactions [1, 10] and rejecting interactions [6, 7, 11] . Our study is the first plant extract–PI trial to utilize a randomized controlled design, and we did not identify an obvious interaction between milk thistle and indinavir. However, by employing this design, we observed that factors other than the plant extract of interest might have important effects on the plasma concentrations of the study medications. In particular, the decline of plasma concentrations of indinavir AUC_{0-8} in the control group relative to that in the milk-thistle group calls into question to what extent time-effects or metabolic factors that were not controlled for may have on the pharmacokinetics of indinavir or other drugs.

A limitation of our study was the small sample size. Although this sample size is larger than in most plant extract–drug interaction trials [12], we are unable to reject the potential of an interaction with milk thistle. Our sample size was further decreased post-trial due to the exclusion of two participant's plasma as a result of contamination. We can, however, be sure that the milk thistle did not cause this as these participants were in the control arm. We attempted to increase the potential power by increasing homogeneity of the participants by including only healthy males between the ages of 18 years and 35 years and dosing with milk thistle for a long-term period (28 days) [13] . A further limitation is the open-label technique that we employed. We decided that placebo should not be used in the trial so as to avoid possible placebo-induced metabolism changes. Since the primary objective of our study was to determine safety, and proving the concept that a control group is needed, we conducted the study on healthy participants. The results of these pharmokinetic studies cannot be directly translated to HIV-positive patients due to the impact of disease state and polypharmacy on drug metablolism [14] . We did not examine the effects of milk thistle on P-glycoprotein (P-gp) in the distribution of indinavir to specific viral sanctuary sites, such as lymph and testes [15]. Further study is required to rule out the potential for such milk thistle/P-gp effects. Several strengths exist in our study. We determined the milk-thistle product to utilize after analysis of five different products and including the target population in the decision making. We extended our trial to include a therapeutic (long-term) dosing period and included a washout period to examine rebound to baseline, as observed in one previously [6] . It is possible that our final phase (phase III) washout period was insufficient duration to determine rebound-to-baseline. However, we additionally exposed our control group to this time period and observed unexpected decreases in plasma concentrations in the control group. As the AUC for indinavir did not return to baseline values in either group, it is possible that indinavir induced transportation by P-glycoprotein (P-gp), thereby facilitating its own elimination [16, 17].

We observed that some individual patients had large decreases in plasma concentrations of indinavir. The greatest decrease was an 81.1% decrease in the AUC (period 1–3) observed in a control participant. Since

Fig. 3 Meta-analysis of milk thistle–indinavir studies

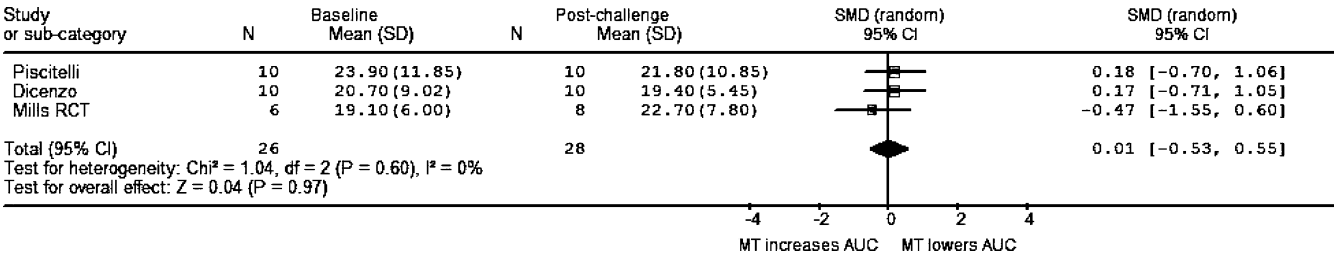

Table 5 Characteristics of three studies included in meta-anlysis

Name, year	*n*	Duration of dosing (days)	Design	Results
Piscitelli, 2002 [6]	10	21	Before-and-after	Milk thistle did not alter significantly the overall exposure of indinavir, as evidenced by an 18% reduction (95% CI, −106% to −70%)
DiCenzo, 2003 [7]	10	14	Before-and-after	Milk thistle resulted in a non-significant reduction of 17% (95% CI, −105% to −71%) $AUC_{0\text{-}8}$
Mills, 2004	16	28	RCT	Milk thistle resulted in a non-significant reduction in comparison with the control arm

these reductions occur in the control group, we cannot make clinical inferences about the potential for milk thistle–indinavir interaction and should be cautious in interpreting clinical inferences made in previous, uncontrolled, milk-thistle studies [6, 7] . A possible explanation for the reduction in indinavir concentrations we observed in the control group is that indinavir, in the dosage used in the study, affects pregnane-x receptors and thereby acts as a key regulator of CYP 3A4 transcription and P-gp [18]. It may be that short exposures to indinavir will affect future pharmacokinetics, as has been observed with nelfinavir, saquinavir and ritonavir [19]. However, to date, no published studies have examined this. Possible explanations for the reduced indinavir concentrations that we observed in the control group require further study.

Our trial additionally indicates caution when extrapolating the conclusions of in vitro studies to clinical settings. Previous in vitro studies had been conducted on milk thistle and concluded that milk thistle had an inhibitory effect on CYP 3A4 [4, 20]. We also observed this effect in our in vitro analysis [5]; however, we did not observe an effect in our clinical trial.

Conclusion

In our trial, we did not observe an interaction between milk thistle and indinavir. Considering the prevalence of herbal medicine use in those living with HIV [21] and the paucity of evidence supporting their use [22], increased efforts are required to design trials that can adequately display or reject plant extract–drug interactions and account for the many complex effects that may occur when plant extracts and drugs are used together.

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