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Relative bioavailability of silybin A and silybin B from two multi-constituent dietary supplement formulations containing milk thistle extract: A single dose study

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Abstract

Purpose—In the current study, we compared the bioavailability between two milk thistle-containing dietary supplements, Product B and IsaGenesis (Isagenix International LLC) in healthy volunteers. Bioavailability between Product B, originally formulated as a powdered capsule, and IsaGenesis, reformulated as a softgel were compared by measuring silybin A and silybin B as surrogate pharmacokinetic markers for differences in absorption and bioavailability.

Methods—For this randomized, open-label, crossover pharmacokinetic study, 12 healthy subjects consumed a single-dose serving of each supplement separated by at least a 7-day washout period. Serial blood samples were obtained at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hour time points and analyzed via LC-MS/MS.

Findings—Rapid absorption and elimination of silybin A and silybin B have been observed after oral administration of both Product B and IsaGenesis. However, the absorption rate and extent, as indicated by mean maximum plasma concentration (C_{max}), and mean plasma AUC, were significantly higher for the IsaGenesis softgel formulation. The dose-corrected mean C_{max} , was 365% and 450% greater for silybin A and B, respectively, relative to powdered Product B. The time to C_{max} , (i.e. T_{max}) was reached, on average, at least 1 hour earlier with IsaGenesis relative to Product B for both silybin A and silybin B.

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Implications—The IsaGenesis softgel formulation provided substantially greater absorption and bioavailability of silybin A and silybin B relative to the powdered Product B supplement.

Keywords

milk thistle; silybin; pharmacokinetics; absorption; formulation

Introduction

Aging is a complex and inevitable biological process characterized by a gradual loss of physiological integrity that is associated with numerous chronically debilitating health effects.¹ The potential function of bioactive phytochemicals as human anti-aging compounds has been strongly promoted with a number of studies suggesting important roles in either disease prevention or a deceleration of age-related processes.² A substantial number of studies have documented the effects of nutraceuticals derived from fruit or plant sources in reducing oxidative damage and promoting healthy aging. Indeed most plant polyphenols exhibit both antioxidant as well as non-antioxidant properties.³ The phytochemicals within nutraceuticals that are generally viewed as potentially beneficial are secondary compounds produced by plants and include broadly, flavonoids, anthocyanin glycosides, triterpenoids, and proanthocyanidins.⁴

The links between dietary habits and health status have been well developed. Consumed fruits and vegetables contain thousands of biologically active phytochemicals that appear to exhibit independent, and potentially, complementary activities to yield antioxidant activity, and potentially modify disease onset or progression.⁵ Numerous studies have suggested that nutraceuticals, defined as nutritional components that impart therapeutic or physiological benefits beyond basic nutritional needs, as well as certain botanical extracts, may potentially promote an array of health benefits including, cognitive health, blood glucose homeostasis, reduce inflammation, prevent cardiovascular disease, inhibit cancer development, and anti-aging effects.^{2,4,5}

Much of the evidence on the beneficial effects of dietary polyphenols is derived from *in vitro* models that frequently use concentrations much higher than those generally achieved through the human diet or through active supplementation (when those concentrations are known). *In vitro* models of bioavailability are useful in predicting/directing *in vivo* bioavailability, but formal clinical study is required to evaluate bioavailability, especially when considering the complex nature of many plant antioxidant-containing matrices, such as dietary supplements containing botanical extracts. The ability of phytochemicals to provide therapeutic benefit is highly dependent on the bioavailability of key active ingredients at the intended target site in adequate concentrations. Unfortunately, many bioactive phytochemicals (e.g. polyphenols) have relatively low bioavailability following oral administration.³ Accordingly, efforts are being made to develop oral dosage formulations which may enhance the bioavailability of bioactive constituents from dietary supplements. These efforts include chemical derivatization, production of nanoparticles, and incorporation of components/carriers that may enhance aqueous solubility.^{3,6}

In the present study, two proprietary blends of natural bioactive plant extracts, amino acids, and vitamins which include milk thistle (*Silybum marinarum*) extract as the predominant botanical component, were studied in healthy volunteers in a randomized, open-label, single-dose crossover pharmacokinetic study (Table 1). These formulations, marketed under the trade names IsaGenesis and Product B, respectively, are purported to promote healthy aging. The Product B formulation was recently reported to significantly increase plasma catalase concentrations compared to placebo treatment in healthy volunteers.⁷ Subsequently, IsaGenesis, a newly-reformulated version was developed as a softgel with fat-soluble components postulated to provide better bioavailability compared to the dry powdered Product B formulation. Because milk thistle extracts were by far the most abundant component in both formulations, and since silybin A and silybin B are found in much higher concentrations in the systemic circulation relative to the amounts of the other flavonolignans (e.g. isosilybin A, silychristin A and B, and silydianin) following oral supplementation with milk thistle extracts,^{8,9} this study utilized the two most abundant flavonolignans found in human plasma, silybin A and silybin B (Figure 1), as surrogate pharmacokinetic markers for differences in the respective formulation's absorption and bioavailability.

Methods and Materials

Study Subjects

Prospective study subjects were recruited into the clinical protocol with a goal of 12 subjects (6 male, 6 female) completing the entire protocol. The study protocol, Informed Consent document, and Health Insurance Portability and Accountability Act (HIPAA) forms and language were reviewed and approved by the University of Florida (UF) Investigational Review Board (IRB) prior to initiating any study procedure or recruitment and all subjects provided written informed consent. The study participants were required to be healthy non-smokers and included both male and female subjects between the ages of 18-50 years. Participating females of child-bearing potential were required to have a negative urine pregnancy test prior to enrollment and avoid pregnancy during study participation. Female subjects were tested prior to each scheduled study day (urine pregnancy test) to provide additional assurance. With the exception of oral contraceptives, no other prescription or OTC medications were permitted. These restrictions also extended to botanical/nutritional supplements, vitamins and "energy drinks". All subjects were required to have a Body Mass Index (BMI) between 18.5 to 28 kg/m² and to have no history of any surgical or medical condition that might interfere with drug absorption, distribution, metabolism, or excretion. Additionally, all subjects were required to have no clinically significant diseases or clinically significant abnormal laboratory values as assessed during a screening medical history, physical exam, and laboratory evaluations.

Clinic Visits and Supplement Administration

Two pharmacokinetic study visits were scheduled and took place at the UF Clinical Research Center (CRC) following informed consent and a satisfactory medical screening. Following an overnight fast, subjects arrived at the UF CRC at approximately 7:30 AM the morning of each of the two supplementation and pharmacokinetic sampling study days.

Each of the two study visits lasted approximately 9 h and were separated by a minimum 7-day wash-out period in between.

After checking in, CRC nursing staff placed an indwelling venous catheter into each subject's arm to facilitate serial blood sampling. Female subjects provided a urine sample for a pregnancy test to be confirmed as negative.

At approximately 8:00 a.m. subjects were administered either two capsules of IsaGenesis or two capsules of Product B depending on the randomization sequence. Two capsules represents a recommended serving of each formulation per product labeling. Capsules were administered with 240 ml of room temperature water which subjects consumed in its entirety. Subjects remained in a fasted state for four additional hours following administration of each formulation to eliminate any potential food effects on absorption. Standard lunches were later provided to the subjects which consisted of a deli-type "sub" sandwich, potato chips and a 12 ounce soda (or bottled water). Subjects consumed an identical meal on their subsequent visit in which the alternate formulation was assessed.

Blood sample collection and processing

Blood samples were collected for silybin A and silybin B analysis via indwelling venous catheters and a total of nine blood samples (~10 ml each) were obtained over the 8-hour period during the respective study days. Specific time points of blood collection occurred immediately prior to the product administration (0 h time point), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 hrs. All samples were drawn in heparinized green-top tubes (BD Vacutainer; Becton Dickinson, Franklin Lakes, NJ) and stored on ice until centrifugation at 4°C. As phytochemicals are variable in their stability within biological matrices, samples were not permitted to remain on ice for longer than 15 minutes prior to centrifugation. Each ~10 ml of whole blood sample yielded ~ 5 ml of plasma following centrifugation. Plasma was then acidified with 10 µl of 1M acetic acid/ml plasma (as a preservative), and transferred to labeled storage tubes frozen immediately at -70°C until analysis by high-performance liquid chromatographic (LC)-tandem mass spectrometric (MS/MS) assay.

Dietary Supplements, Chemicals and Standards

Milk thistle containing oral dosage forms Product B (LOT# 041403615) containing 125 mg/capsule of milk thistle extract per capsule (80% silymarin), and the IsaGenesis (LOT# 025400816) containing 160 mg/softgel capsule (55% silymarin) were each from a single lot source, and provided by the study sponsor, Isagenix International, LLC. IsaGenesis is manufactured at Best Formulations (City of Industry, CA) while Product B was manufactured by Arizona Nutritional Supplements (Chandler, AZ). Relative to Product B, a major pharmaceutical difference in IsaGenesis was the addition of vegetable oils and phospholipid-50 (50% solution of phosphatidylcholine) to increase absorption and bioavailability of silybin. The entire content of each formulation is provided in Table 1. Both formulations had previously passed the USP 2040 disintegration and dissolution test of dietary supplements.¹⁰

Authentic analytical reference standards of silybin A and silybin B were obtained from Phytolab GmbH & Co. (Vestenbergsgreuth, Germany). The internal standard (IS) naringenin was purchased from SAFC Supply Solutions (St. Louis, MO). Liquid chromatography-mass spectrometry grade methanol, ammonium acetate, formic acid, and methyl tert-butyl ether were all purchased from Sigma-Aldrich (St. Louis, MO).

Analytical Methods

Analysis of Silybin A and B from Oral Dosage Forms

Prior to the pharmacokinetic study, an analysis and comparison of the silybin A and silybin B content of the two respective formulations was undertaken. In brief, the content of two capsules (1 serving) of each formulation were weighed, placed into a 10 ml volumetric flask, and dissolved in 100% analytical grade methanol. A 1 ml aliquot of the resulting solution was removed and further diluted to a proper concentration suitable for LC-MS/MS analysis. Naringenin served as the internal standard (IS).

Analysis of Silybin A and B from Human Plasma

A LC-MS/MS assay was utilized in our laboratory for simultaneously analyzing the nonconjugated flavonolignans silybin A and silybin B. This method was largely based upon our previously validated method for analyzing multiple flavonolignans simultaneously in human plasma.¹¹

Instrumentation—The LC-MS/MS analysis was performed on a Shimadzu HPLC system (Shimadzu, Tokyo, Japan) including two pumps (LC-10ATvp), an autosampler (SIL-10ADvp), and a system controller (SCL-10Avp) coupled to an Applied Biosystems-Sciex API 3000 triple quadrupole mass spectrometer (Foster City, CA, USA).

Method—The method was based upon that of Brinda and coworkers (2012).¹¹ In brief, 200 μ l of plasma, 100 ng/ml naringenin and 2 ml of methyl tert-butyl ether were added. The samples were shaken at 200 cycles/min for 10 min and then centrifuged at 3000 *g* for 10 min at room temperature. The organic phase was then transferred to clean glass tubes. The supernatant was evaporated to dryness under a gentle stream of nitrogen at 40°C, and the remaining residue was reconstituted with 100 μ l of mobile phase, 20 μ l of which were injected for analysis. Measurement of individual silymarin constituents (i.e. silybin A and silybin B) was achieved by running samples through a C18 guard column (4 mm $\times$ 20 mm, SecurityGuard, Torrance, CA) before separation on a Phenomenex Luna 5 μ m C18 column (100A 250 $\times$ 2 mm, 5 μ m, Torrance, CA). The mobile phase consisted of 50% methanol with 0.1% formic acid and 50% water containing 0.1% formic acid and 10 mM ammonium acetate, and was delivered at a low rate of 0.25 ml/min. The MS was operated in negative ion mode using turbo electrospray ionization. The MS tuning parameters were optimized for each analyte by infusing 0.1 mg/ml of each compound dissolved in mobile phase at a low rate of 20 ml/min. The following parameters were used for the MS analysis: curtain gas, 8 psi; nebulizer gas (gas 1), 12 psi; CAD gas, 6 psi; TurboIonSpray (IS) voltage, -4500V; entrance potential, -10V; collision cell exit potential, -6 V; declustering potential, -80 V; collision energy, 40 eV for *m/z*: 481.2>125 and 26 eV for *m/z*: 271.1>151.0; source

temperature, 400 °C; and dwell time, 200 ms. The following transitions were monitored in the Multiple Reaction Monitoring mode: silybin A, silybin B, m/z 481.2 > 125; and naringenin (IS), m/z 271.1 > 151.0. Data were acquired and analyzed by AB Sciex Analyst software, version 1.4.2 (AB Sciex, Toronto, Canada).

The lower limit of quantification was 1 ng/ml for both silybin A and silybin B. Calibration curves were linear over the range of 1 to 100 ng/ml for both analytes ($r^2 > 0.99$). The intra- and inter-day accuracies were 98.4-106.5% and 94.2-103.42%, respectively. The intra- and inter-day precision was less than 13.95%.

Pharmacokinetic Analysis

Nine plasma concentration values were determined in 12 healthy volunteers participating in a single dose randomized crossover pharmacokinetic study after alternately receiving a single oral administration of either two capsules of Product B formulation containing 125 mg of milk thistle extract per/capsule or two softgel capsules of IsaGenesis formulation containing 160 mg milk thistle extract per/capsule on alternating study visits.

Plasma determinations of silybin A and silybin B over an eight-hour period were made via an LC-MS/MS assay as described above. The pharmacokinetic parameters of silybin A and silybin B were estimated by a non-compartmental analysis (NCA) using WinNonlin 6.1 (Pharsight, Mountain View, CA). The maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) were obtained directly from the plasma concentration-time data. The terminal elimination rate constant (λ_z) was estimated by linear least-squares regression of the terminal portion of the plasma concentration time curve, and the elimination half-life ($t_{1/2}$) was then calculated using the formula $t_{1/2} = 0.693/\lambda_z$. The area under the plasma concentration-time curve (AUC) from time 0 to infinity (AUC_{inf}) and AUC from time 0 to the last measurable concentration (AUC_{last}) were calculated according to the linear trapezoidal rule. Regarding relative bioavailability, the following calculation as used:

$$\text{Relative Bioavailability(F)} = (\text{AUC/Dose})_{\text{IsaGenesis}} / (\text{AUC/Dose})_{\text{Product B}} * 100\%$$

Results are presented as median (range) for T_{max} and mean $\pm$ S.D. for all other pharmacokinetic parameters.

In order to preliminarily assess potential gender differences in peak and systemic exposure of silybin isomers, Student's t-test was applied to analyze the log-transformed C_{max} and AUC_{last} of silybin A and silybin B after oral administration of either Product B or IsaGenesis between male versus female subjects. $P < 0.05$ is considered statistically significant.

Study Results

Formulation Analysis

The results of the capsule analysis are presented in Table 2. These values were used in terms of correcting overall silybin exposure to the study subjects. Both formulations contained approximately equal amounts of the silybin A and silybin B isomers as is typical of silymarin extracts.

Research Subjects

Twelve healthy volunteers (six men and six women) aged 19–34 years (24.1 ± 3.7 years; weight, 67.9 ± 11.6 kg; mean $\pm$ S.D.) completed the entire protocol. Both formulations were well-tolerated. No subjects were discontinued from or withdrew from the study. No subject experienced any unanticipated adverse events related to either studied formulation or any study-related procedure.

Silybin A and B Pharmacokinetics

Pharmacokinetic analysis indicated that after oral administration of either Product B or IsaGenesis, the flavonolignans silybin A and silybin B were rapidly absorbed and eliminated. The associated pharmacokinetic parameter estimates of silybin A and silybin B generated in this study following a single oral dose (serving) of Product B and IsaGenesis are presented in Table 3. Mean plasma concentrations vs time profiles of silybin A and silybin B are presented in Figures 2 and 3 respectively, while Figure 4 depicts the mean plasma concentration vs time profiles of total silybins (i.e. silybin A+B).

Since the two respective formulations contained unequal total amounts of silybins, and to provide a further comparison between these two formulations, Figures 2–4 also depict a *third theoretical profile* in which the IsaGenesis formulation was ‘dose adjusted’ by multiplying the mean values by factor of 0.88 (176/200 based on different silymarin exposure) to approximate the profile of the newer formulation were it delivered in the exact dosage strength as Product B (Table 1). This adjustment was made assuming linearity of silymarin A and B absorption and disposition.

Silybin A and silybin B from the IsaGenesis softgel formulation displayed a distinctly different pharmacokinetic profile from that of Product B formulation over the 8-hour blood sampling period, which is depicted in Figures 2 and 3. The IsaGenesis softgel formulation exhibited a more rapid rise in plasma concentration and attained higher peak concentrations (i.e. $C_{\max}$) of silybin A and B over the course of the first 6 hours when compared with Product B. Moreover, the IsaGenesis softgel formulation exhibited an earlier median time to maximum plasma concentration ($C_{\max}$) or $T_{\max}$. The $T_{\max}$ values were 2.0 h for IsaGenesis vs 3.0 h for Product B for both silybin isomers (Table 3). Additionally, despite similar content of total silybins (per measured capsule content of silybin A and silybin B), the IsaGenesis formulation resulted in a 365% and 450% greater $C_{\max}$, for silybin A and silybin B, respectively, relative to Product B. Indeed, normalized to dose, silybin A $C_{\max}$ was 1.17 ± 0.67 vs 0.32 ± 0.23 ng/ml/mg and silybin B was 0.72 ± 0.57 vs 0.16 ± 0.14 ng/ml/mg, respectively for IsaGenesis vs Product B. Regarding *relative bioavailability* (F),

the values in Table 3 indicate that absorption with the softgel was 284% (silybin A) and 334% (silybin B) greater than with the Product B formulation.

As with most pharmacokinetic studies of botanical formulations, *inter*-individual differences and variability in concentration-time curves were noted. Labeled Figures 4–8 below are boxplots illustrating the variability observed in the two formulations with respect to AUC_{last} and C_{max} of both silybin A and silybin B. These plots present the minimum, first quartile, median, third quartile, and maximum values. With regard to *intra*-individual variability, the subject design did not permit an evaluation since each subject received each formulation only once. However, it was noted that in subjects who exhibited lower concentrations of silybins and lower overall absorption did so with both formulations suggesting some suggesting some gastrointestinal differences rather than formulation issues.^{12,13}

Finally, no statistically significant difference in AUC_{last} and C_{max} of either silybin A or silybin B between male and female subjects could be observed in the study ($P > 0.05$; Supplementary table 1). However, due to the limited sample size and large inter-individual variability, gender differences in the pharmacokinetics of silybin isomers cannot be determined or ruled out.

Discussion

It is essential to understand the bioavailability (i.e. rate and extent of absorption) of phytochemicals administered as oral supplements in order to evaluate and interpret biological activities and potential health benefits in humans. This information is critical in order to determine if promising results obtained from *in vitro* studies are likely to be achieved *in vivo* i.e. there must be sufficient delivery or penetration of the putative active constituent(s) to their target site of biological activity.

An accurate assessment of bioavailability and distribution is lacking for almost all commercial formulations and relatively few formal pharmacokinetic studies have been performed to date in human subjects. Significant pharmacokinetic variability among the many commercially available milk thistle extracts has been documented, and the bioavailability of most oral formulations is generally regarded as poor and highly variable.⁹ Major reasons cited for the low bioavailability of silymarin constituents include extensive pre-systemic (i.e. first-pass) conjugative metabolism, poor permeability across intestinal cells, and low aqueous solubility.

A study of the comparative pharmacokinetics of a single oral administration of two similar multi-constituent dietary supplement formulations was completed. Subjects were randomly assigned to receive on alternate visits either two capsules of Product B formulation which contained 125 mg of milk thistle extract per/capsule or two softgel capsules of the IsaGenesis formulation which contained 160 mg milk thistle extract per/capsule. The flavonolignans silybin A and silybin B served as surrogate markers for constituent absorption and relative bioavailability of the formulations. Additionally, consistent with other published reports assessing silybin pharmacokinetics in human subjects, the peak and systemic exposure (i.e. C_{max} and AUC_{inf}) were significantly higher for silybin A compared

with silybin B.^{8,9} These observed differences are attributed to the known stereoselective metabolism (i.e. glucuronidation) of the silybins with a greater preference for silybin B.¹⁴

Pharmacokinetic analysis indicated that in general, silybin A and silybin B were rapidly absorbed and eliminated with both formulations. Overall, the softgel formulation, IsaGenesis, appeared to be absorbed significantly better than the comparator, Product B, with respect to silybin A and silybin B. Indeed, despite very similar doses (i.e. per measured content of silybin A and silybin B), the IsaGenesis formulation resulted in 365% and 450% higher mean C_{max} values, respectively, relative to the Product B formulation. Furthermore, it was noted that the C_{max} values attained with the IsaGenesis formulation for silybin A were in the range of values we have previously determined for a single capsule of a well characterized silymarin formulation.⁹ Regarding T_{max} , on average, both silybin A and silybin B reached the C_{max} at least 1 hour earlier with the IsaGenesis formulation relative to Product B (Table 3). The calculated $t_{1/2}$ of the silybins appeared to differ between the two formulations. Both silybin A and silybin B have a shorter $t_{1/2}$ with the IsaGenesis formulation relative to Product B (Table 3) and the reasons for this are unclear.

Inter-individual pharmacokinetic variability was observed with both formulations. From the box plots presented in Figures 4–8, there appears to be a greater amount of inter-subject variability with the IsaGenesis formulation.

Although the oral bioavailability of certain natural antioxidants, such as the flavonolignans is relatively low compared to concentrations that may be utilized in *in vitro* assessments of biological activities, there remains the possibility that they may exert their biological activity at relatively low systemic concentrations.

There are some limitations to the present study. First, the respective dosage formulations were not single botanical extract formulations as they contained multiple botanical constituents from different plant sources. Secondly, the two dosage forms had similar but unequal amounts of silymarin content (silybin A and silybin B) as well as other botanical extracts. However, the unequal amounts of silybin A and silybin B, administered in the compared formulations were dose corrected for purposes of pharmacokinetic comparisons.

Scant research is available relative to the pharmacokinetics of the silybins in humans when administered as part of a multi-constituent supplement and the potential influence of other included phytochemicals on the disposition of silybin A and B remains an open question. The possibility of competitive or synergistic effects on absorption and metabolism among various phytochemicals administered as part of multi-constituent formulation versus the administration of a single extract or extract component from a natural product has been noted.¹⁵ Additionally, although the IsaGenesis formulation clearly appeared to be better absorbed with respect to silybin A and silybin B systemic exposure, it must be emphasized that these findings cannot simply be generalized to the many other constituents found within either of the two multi-constituent formulations without directly assaying plasma for representative constituents.

Flaxseed oil and phosphatidylcholine were excipient components of the IsaGenesis softgel formulation but not the Product B formulation. Flaxseed oil was added as an n-3 fatty acid

source and potential diluent for the fat-soluble bioactives but was not included with the specific intent of enhancing bioavailability. Regarding the phosphatidylcholine content, this was not present as a silybin-phosphatidylcholine complex as has been described for some primary silymarin formulations.¹⁶ However, recognizing the abundance of this phospholipid in cellular membranes and the established role of phospholipids as excipients for various pharmaceutical applications including as emulsifiers, wetting agents, and solubilizers¹⁷, it was felt that its inclusion via a microcoating manufacturing process might enhance the absorption of fat soluble phytoconstituents. Although the IsaGenesis formulation did appear to provide greater overall exposure to the silybins, whether this was due to phosphatidylcholine inclusion remains a matter of speculation at this point that would require further study.

In summary, greater overall systemic exposure to both silybin A and B occurred with the IsaGenesis softgel formulation relative to the dry-powdered Product B formulation. However, potentially more inter-individual variability in silybin concentrations was also observed. Further investigation is required to study the potential antioxidant effects of the multi-constituent oral supplement IsaGenesis in patients exhibiting elevated levels of oxidative stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

John W. Anderson, was the formulator and inventor of both formulations assessed in this study.

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Figure 1.
Structures of Silybin A and Silybin B.

Figure 2. Mean plasma concentrations of silybin A versus time profiles after oral administration of single doses of Product B and IsaGenesis in 12 healthy volunteers.

Figure 3. Mean plasma concentrations of silybin B versus time profiles after oral administration of single doses of Product B and IsaGenesis in 12 healthy volunteers.

Figure 4. Mean plasma concentrations of silybin (A+B) versus time profiles after oral administration of single doses of Product B and IsaGenesis in 12 healthy volunteers.

Figure 5.
Box plots of $AUC_{last}/Dose$ ($h \cdot ng/ml/mg$) of Silybin A.

Figure 6.
Box plots of $C_{max}/Dose$ (ng/ml/mg) of Silybin A.

Figure 7.
Box plots of $AUC_{last}/Dose$ ($h \cdot ng/ml/mg$) of Silybin B.

Figure 8.
Box plots of $C_{max}/Dose$ (ng/ml/mg) of Silybin B.

Table 1

Formulation Comparison and Content

IsaGenesis (2 softgels)		Product B (2 capsules)	
Ingredient	Amount	Ingredient	Amount
Milk Thistle (<i>Silybum marianum</i> PE 55% silymarin)	320 mg	Milk Thistle (<i>Silybum marianum</i> PE 80% silymarin)	250 mg
Pomegranate Juice (4:1 PE)	30 mg	Pomegranate (<i>punica granatum</i> PE)	29 mg
Grape Seed (80% oligomeric proanthocyanidins)	20 mg	Grape Seed (80% oligomeric proanthocyanidins)	20 mg
Turmeric (95% curcuminoids)	64 mg	Turmeric (95% curcuminoids)	20 mg
Ashwagandha (<i>Withania somnifera</i> 4:1 PE)	27.5 mg	Ashwagandha (<i>Withania somnifera</i> 4:1 PE)	50 mg
Bacopa (<i>Bacopa monnieri</i> whole plant 25:1 PE)	10 mg	Bacopa (<i>Bacopa monnieri</i> PE 50%)	9 mg
Green Tea (35% EGCG)	64 mg	Green Tea (98% polyphenols)	35 mg
Horny Goat Weed (5:1 PE)	60 mg	Horny Goat Weed	30 mg
Knotweed root	62 mg	–	
R- α -Lipoic Acid	12 mg	–	
Black Tea (5:1 PE)	4 mg	Black Tea (PE 50% polyphenols)	4 mg
White Tea (30% EGCG)	4 mg	White Tea (PE 90% polyphenols)	4 mg
Blueberry	18 mg	Blueberry	7 mg
Raspberry	10 mg	Red Raspberry	7 mg
N-Acetyl Cysteine	18 mg	–	
L-Glutathione	10 mg	–	
Quercetin	8 mg	–	
Bilberry	14 mg	Bilberry	11 mg
Panax Ginseng (25:1 PE)	10 mg	Panax Ginseng (Root PE 80% ginsenosides)	12 mg
<i>Mucuna Pruriens</i> (4:1 PE)	6 mg	–	
Harada (<i>Terminalia chebula</i> fruit)	8 mg	Harada (<i>Terminalia chebula</i> fruit)	8 mg
Boswellia (20:1 PE)	18 mg	Boswellia (PE 65% boswellic acid)	7 mg
Maca	6 mg	Maca	7 mg
Shilajit	6 mg	Shilajit (20% fulvic acid)	5 mg
–		Resveratrol	28 mg
Ascorbic Acid	13 mg	Ascorbic Acid	7.5 mg
Acacia Bark (12:1 PE)	4 mg	–	
Goldthread (5:1 PE)	6 mg	–	
α -tocopherol	5 IU	–	
Vitamin B ₁₂	12 μ g	–	
Canola Lecithin	10 mg	–	
Vitamin A	2 mg	–	
Flaxseed Oil	250 mg	–	
Lutein	1 mg	–	
Zeaxanthin	.33 mg	–	

IsaGenesis (2 softgels)		Product B (2 capsules)	
Ingredient	Amount	Ingredient	Amount
Phosphatidylcholine	200 mg	–	

EGCG= Epigallocatechin gallate, PE=Pharmaceutical Equivalents

Table 2

Comparison of Silybin A and Silybin B amounts in the two formulations Each sample is taken as 1/10th from the mixture of two capsules (1 serving) and diluted (10,000-fold) to a concentration in LC-MS/MS detection range.

Formulation	Silybin A content (mg)	Silybin B content (mg)	Silybin A+B content (mg)
IsaGenesis (Softgel)	55.1	87.5	142
Product B	48.7	80.3	129

Table 3

Pharmacokinetic parameter estimates of silybin A and silybin B after single oral doses of Product B and IsaGenesis. The T_{max} values are expressed as median (range) and others are the mean $\pm$ S.D. (n=12).

Pharmacokinetic Parameter	Silybin A Product B	Silybin A IsaGenesis	Silybin B Product B	Silybin B IsaGenesis
Dose (mg)	48.7	55.1	80.3	87.5
T_{max} (h)	3.0 (1.5-6)	2.0 (1-4)	3.0 (1.5-4)	2.0 (0.5-4)
$t_{1/2}$ (h)	1.98 ± 1.64	1.30 ± 0.32	1.82 ± 1.20	1.28 ± 0.34
C _{max} (ng/ml)	15.69 ± 11.41	64.42 ± 37.09	12.86 ± 11.39	62.83 ± 49.84
$C_{max}/Dose$ (ng/ml/mg)	0.32 ± 0.23	1.17 ± 0.67	0.16 ± 0.14	0.72 ± 0.57
AUC_{last} (ng×h/ml)	37.17 ± 30.27	118.78 ± 55.88	30.15 ± 31.59	110.95 ± 74.26
$AUC_{last}/Dose$ (ng×h/ml/mg)	0.76 ± 0.62	2.16 ± 1.01	0.38 ± 0.39	1.27 ± 0.85
$AUC_{INF}/Dose$ (ng×h/ml/mg)	0.96 ± 0.62	2.24 ± 1.04	0.52 ± 0.40	1.31 ± 0.84
F (IsaGenesis/Product B)	284%		334%	

Regarding relative bioavailability (F) the values in Table 3 of 284% (silybin A) and 334% (silybin B) represent the relative bioavailability of the respective silybin isomers in IsaGenesis (softgel) compared to that in Product B formulation. Given silybin isomers AUC_{last}/AUC_{inf} in Product B were less than 80%, dose corrected AUC_{last} was adopted in the estimation of relative bioavailability.

$$\text{Relative Bioavailability}(F) = \left(AUC_{last}/Dose \right)_{\text{IsaGenesis}} / \left(AUC_{last}/Dose \right)_{\text{Product B}} * 100\%$$