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RESEARCH ARTICLE

Pharmacokinetics of xanthohumol and metabolites in rats after oral and intravenous administration

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Scope: Xanthohumol (XN), a dietary flavonoid found in hops, may have health-protective actions against cardiovascular disease and type 2 diabetes. Yet, there are limited data on the pharmacokinetics (PK) of XN. This study provides PK parameters for XN and its major metabolites in rats.
Methods and results: A PK study was conducted in male jugular vein-cannulated Sprague–Dawley rats. Rats ($n = 12$/group) received an intravenous (IV) injection (1.86 mg/kg BW) or an oral gavage of a low (1.86 mg/kg BW), medium (5.64 mg/kg BW), or high (16.9 mg/kg BW) dose of XN. Plasma samples were analyzed for XN and its metabolites using LC-MS/MS. The maximum concentration (C_{max}) and area under the curve ($AUC_{0\text{-}96\,h}$) of total XN (free and conjugated) were 2.9 ± 0.1 mg/L and 2.5 ± 0.3 h* mg/L in IV group, 0.019 ± 0.002 mg/L and 0.84 ± 0.17 h* mg/L in the oral low group, 0.043 ± 0.002 mg/L and 1.03 ± 0.12 h* mg/L in the oral medium group, and 0.15 ± 0.01 mg/L and 2.49 ± 0.10 h* mg/L in the oral high group.
Conclusion: The bioavailability of XN is dose-dependent and approximately 0.33, 0.13, and 0.11 in rats, for the low-, medium-, and high-dose groups, respectively.

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Bioavailability / Flavonoids / Hops / Pharmacokinetics / Xanthohumol

1 Introduction

The flowers of the female hop plant (*Humulus lupulus* L.) are used in the brewing industry to add bitterness and flavor to beer. The bitter principles of the hop flowers (commonly referred to as 'hops' or 'hop cones') are prenylated acylphloroglucinol derivatives in a biogenetic sense, and they can be classified as humulones (α-bitter acids) or lupulones (β-bitter acids). Structurally related to the bitter acids are the prenylated flavonoids, which are produced by hops in specialized glands together with the bitter acids and secreted as a sticky resin called 'lupulin'. The principal prenylated flavonoid of hops is xanthohumol (XN) [1], a yellow substance also found in beer [2], which may have health-promoting properties as an anti-oxidant [3], an anti-inflammatory agent [4, 5], a cancer chemopreventive agent [6–13], a modulator of the immune system [14], an anti-microbial agent [15], and as an inhibitor of osteoporosis in postmenopausal women [16].

Recent evidence suggests that dietary bioactives could aid in the prevention and/or halt the progression of several chronic diseases [17–20]. Nearly, 50 million Americans have metabolic syndrome and are at increased risk for cardiovascular disease and type 2 diabetes [21]. In particular, daily oral administration of XN for one month has been shown by Nozawa [22] to lower plasma triacylglycerols and glucose levels in KK-A^y mice, a model for obesity and type 2 diabetes. Nozawa's study also demonstrated a decrease in wet epididymal weight and an increase in plasma adiponectin levels. In a study conducted by Mendes et al. [23], XN reduced differentiation, decreased proliferation, and increased apoptosis in the murine preadipocyte cell line, 3T3-L1,

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Abbreviations: **6PN,** 6-prenylnaringenin; **8PN,** 8-prenylnaringenin; **BW,** body weight; **DHC,** 4,2′-dihydroxychalcone; **IV,** intravenous; **IX,** isoxanthohumol; **PK,** pharmacokinetics; **SRM,** selective reaction monitoring; **XN,** xanthohumol

at concentrations in the range of 1–10 μM. Similar effects of XN were observed by Rayalam et al. [24] and also by Yang et al. [25], who found that the XN-induced apoptosis of 3T3-L1 cells resulted from increased production of reactive oxygen species. In cultured hepatoma HepG2 cells, XN inhibited triacylglycerol synthesis by 62% at 15 μM and dose-dependently decreased apolipoprotein B secretion by 13–43% at 5−15 μM [26]. The decrease in triacylglycerol synthesis was attributed to the inhibition of cellular diacylglycerol acyltransferase activity [26]. In vitro and in vivo studies have also shown that XN may reduce the risk for non-alcohol steatohepatitis, a serious liver condition associated with obesity and type 2 diabetes [27]. Dorn et al. observed inhibition of hepatic inflammation and fibrosis with 3-wk feeding of XN (1% w/w XN in diet) in BALB/c mice [27]. Taken together, these studies suggest that XN may exert beneficial effects in dyslipidemia and its complications.

The question that remains to be answered is whether sufficient plasma and tissue levels of XN or its active metabolites can be reached by oral intake of XN in order to obtain the biological effects observed in cell culture studies. In vitro and animal studies have established that the chalcone XN is non-enzymatically converted into its flavanone isomer, isoxanthohumol (IX), and enzymatically into 6- and 8-prenylnaringenin (6PN and 8PN) (Fig. 1). The conversion of IX into 8PN is mediated by hepatic CYP1A2 [28] or by gut microflora enzymes [29–31]. XN and its metabolites are found in the free form or as conjugates (mainly as glucuronides and sulfates) [32–34]. Minor metabolites of XN include chalcones or flavanones with oxygenation of the prenyl substituent, the olefin carbons between the A- and B-rings, and the B-ring itself [35, 36].

Very few studies have been published on the quantitative aspects of XN absorption, distribution, metabolism, and excretion in vivo. Hanske et al. [37] administered XN intragastrically (48 μmol/kg body weight = 17 mg/kg BW) to germ-free and human microbiota-associated Sprague–Dawley rats. The microbiota adequate rats showed maximal blood concentrations of 0.65 μM (free XN), 0.11 μM (conjugated XN), 1.04 μM (free IX), and 4.87 μM (conjugated IX), while 8PN was not detected. The recovery of the administered XN dose from the urine and the feces amounted to 4.2% in the form of XN, IX, 8PN, and their conjugates [37]. Other studies also indicate low recovery of XN and its metabolites. Bolca et al. [31] reported that the mean recovery of free and conjugated XN was 0.32% from the 24 h urine of post-menopausal women after 5 days of daily intake of 1.38 mg XN. These data show that XN is absorbed and metabolized in vivo, but the bioavailability of XN has not yet been established in quantitative terms.

The aim of the present study was to determine the basic aspects of the absorption, distribution, and metabolism of XN in male jugular vein-cannulated Sprague–Dawley rats. A single-dose pharmacokinetics (PK) study was conducted at three oral dose levels and one intravenous (IV) dose level to determine bioavailability and dependence of PK parameters on dose level. The PK parameters from this study make it possible to estimate plasma levels of XN and its metabolites (IX, 6PN, and 8PN) for any dosage regimen at steady-state, a necessary step in the evaluation of potential of XN as an agent in the prevention/treatment of dyslipidemia and possibly other metabolic disorders.

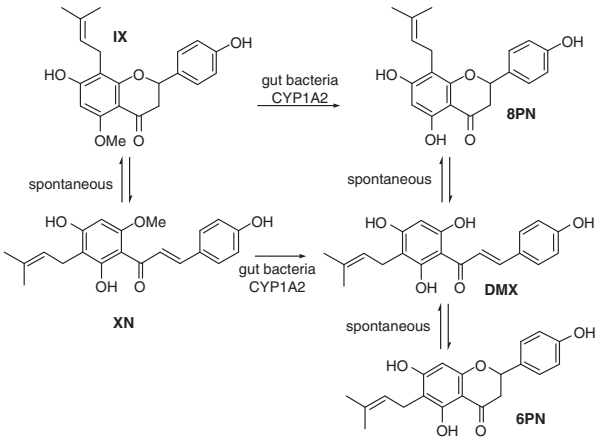

Figure 1. Metabolic conversion of XN into desmethylxanthohumol (DMX) and 6- and 8-prenylnaringenin (6PN and 8PN). XN is spontaneously converted into isoxanthohumol (IX) by intramolecular Michael addition. 6-Prenylnaringenin (6PN) is formed from DMX. 8-Prenylnaringenin is formed from DMX or from IX by CYP-mediated demethylation [28, 31].

2 Materials and methods

2.1 Animals

Six-week-old male Sprague–Dawley rats were purchased from Harlan (Livermore, CA, USA) and underwent jugular catheter implantation surgery two days before shipment. Rats were maintained on an American Institute of Nutrition rodent diet (AIN 93G) [38] with corn oil replacing soy oil and deionized water ad libitum throughout the study. Animals were housed in individual cages in temperature- and humidity-controlled rooms with a 12:12 on–off light cycle. All procedures were approved by Oregon State University's Institutional Animal Care and Use Committee (Protocol 3689).

2.2 Treatment groups

After a two-day acclimation period, animals were divided into four treatment groups ($n = 12$/group): IV (1.86 mg XN/kg BW), oral low (1.86 mg XN/kg BW), oral medium (5.64 mg XN/kg BW), and oral high (16.9 mg XN/kg BW). These oral dose levels were selected to correspond to oral doses of 20, 60, and 180 mg XN in humans by using allometric interspecies scaling (Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078932.pdf)

[39]. Animals were selected to ensure similar body weight average across treatment groups.

2.3 Dose and sampling

XN powder was a gift from Anheuser-Busch (St. Louis, MO, USA) and checked for identity and purity (>99%) by ^{1}H NMR and HPLC with photodiode detection. For each oral dose level, appropriate amounts of XN powder were dissolved in a self-emulsifying isotropic mixture of oleic acid, propylene glycol, and Tween 80 (0.9:1:1 by weight). After a 12-h fast, animals received a single oral gavage solution (0.5 mL) at three dose levels (1.86, 5.64, or 16.9 mg/kg BW). The animals in the IV group were given an IV injection (1.86 mg/kg BW) of XN dissolved in propylene glycol. The IV dose was administered through the jugular catheter. Before each blood draw, the flushing solution was removed from the catheter and blood was drawn to the end of the catheter before a clean sterile needle was placed on for blood collection. Blood (0.3 mL) was drawn via jugular catheter from each rat at the following time points: 0, 0.2, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 48, 72, and 96 h. After each blood draw, the jugular catheter was flushed with 0.3 mL heparinized saline (20 U/mL) to maintain constant blood volume. Food was returned to animals after the 4 h blood collection. Blood samples were placed in collection tubes coated with lithium heparin (Microvette CB 300, Sarstedt, Newton, NC, USA) and stored on ice immediately after collection. Samples were centrifuged for 10 min at 2000 × *g* (VWR Microcentifuge Galaxy 7D, VWR, Radnor, PA, USA). Plasma was stored at −80°C until analysis for XN and its metabolites.

2.4 Sample preparation

Aliquots (10 μL for IV and 25 μL for oral groups) of plasma in duplicate were diluted with sodium acetate buffer (0.1 M, pH = 4.7), spiked with of 4,2′-dihydroxychalcone (DHC) (2.4 ng in methanol) (Indofine Chemical, Hillsborough, NJ, USA) as the internal standard, and treated with 600 U of *Helix pomatia* hydrolases dissolved in sodium acetate buffer (Sigma, St. Louis, MO, USA) for 3 h at 37°C in a total volume of 600 μL to convert glucuronide and sulfate conjugates into their free aglycone forms. Incubation solutions were extracted thrice with diethyl ether (1.0 mL) and centrifuged for 1 min at 8500 × *g* (VWR Microcentifuge Galaxy 7D, VWR). The combined ether extracts were taken to dryness with a stream of nitrogen gas using a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA). The residues were dissolved in 0.1 mL of methanol containing 0.1% formic acid, briefly vortexed (10 s), and sonicated (30 s), and analyzed directly by LC-MS/MS.

XN standard was obtained by isolation from hops and IX by isomerization of XN [1, 2]. Standards of 6PN and 8PN were obtained by chemical prenylation of naringenin (Sigma) and chromatographic separation of the regioisomers [40]. Calibration curves were prepared by spiking blank rat plasma with known concentrations of XN, IX, 6PN, 8PN, and the internal standard, DHC, using 7–10 concentration levels covering the entire concentration range for all analytes in the samples. The plasma-based calibration samples were treated the same as the samples obtained from dosed animals. The method was validated for precision (12% RSD or better), accuracy (90–110% recovery of standards from blank plasma) and for limit of quantitation (0.4–0.5 nM, depending on analyte).

2.5 MS

LC-MS/MS was performed on an Applied Biosystems 4000 QTRAP hybrid linear ion trap-triple quadrupole instrument (AB Sciex, Concord, ON, Canada) operated at a source temperature of 600°C with a needle voltage of −4500 kV. Nitrogen was used as the source gas, curtain gas, and collision gas. Selected reaction monitoring (SRM) experiments were conducted at collision energies ranging from −25 to −40 eV. Concentrations were calculated using the internal calibration method and Analyst Software (Analyst 1.5, AB Sciex).

A Shimadzu Prominence HPLC system (Shimadzu, Columbia, MD, USA), consisting of two LC-20AD pumps, a DQU-20A$_5$ degasser, and an SIL-HTC autosampler, equipped with switching valves, were used for all chromatography. Chromatographic separations of XN and its metabolites were achieved on a 2 × 50 mm Zorbax 300SB C8 column (Agilent, Santa Clara, CA, USA) eluted with a gradient of 25–60% solvent B (0.1% formic acid in ACN) in solvent A (aqueous 0.1% formic acid) in 2.6 min at a flow rate of 0.5 mL/min after an initial 1.4 min at 25% solvent B. The column was washed with 100% solvent B for 1.4 min and re-equilibrated at 25% solvent B for 9 min prior to each injection. Precursor → product ion transitions for SRM were developed using standards. SRM transitions used for quantitation included: 353 → 119 for IX and XN, 339 → 219 for 8PN and 6PN, and 239 → 119 for DHC (Fig. 2).

2.6 PK model and parameters

Mean plasma concentration–time profiles for XN and its metabolites (IX, 6PN, and 8PN) were generated using the GraphPad Prism software (version 4.03, San Diego, CA, USA). The concentration–time profiles were fitted to different compartmental models and the goodness of fit was determined based on Akaike and Schwarz criteria values [41] using WinNonlin software (version 5.0.1; Pharsight, Sunnyvale, CA, USA). A two-compartment model was selected to describe the data. The models for oral (Eq. 1) and IV (Eq. 2) administration were defined by the following equations:

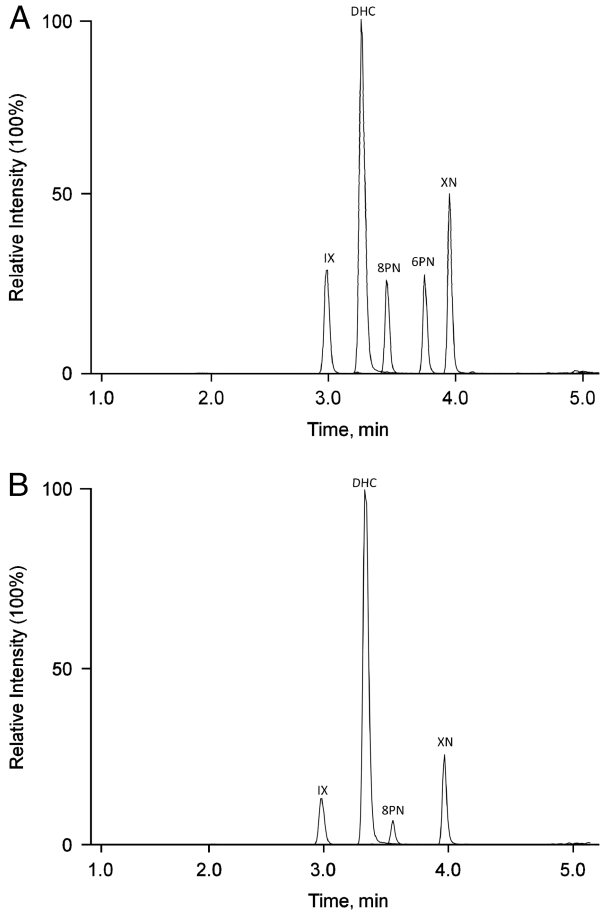

Figure 2. Representative LC-MS chromatograms of (A) a 0.2 μM standard and (B) a 24 h plasma sample from an oral high- (16.9 mg/kg BW) dosed animal. The standard contained xanthohumol (XN) and its metabolites (isoxanthohumol, IX; 8-prenylnarigenin, 8PN; and 6-prenylnarigenin, 6PN) as well as an internal standard (4, 2′-dihydroxychalcone, DHC).

Oral administration:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-k_a t} \quad (1)$$

Intravenous administration:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (2)$$

where α is the first-order distribution rate constant, β is the first-order elimination rate constant, and k_a is the absorption rate constant; *A*, *B*, and *C* are the coefficients for distribution, elimination, and absorption phases, respectively. *C*(*t*) represents the concentration of XN at a given time, *t*. Maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined from the plasma concentration–time curves. Optimized estimates for the model parameters were obtained using nonlinear regression analysis and WinNonlin software (WinNonlin 5.0.1; Pharsight). Estimates were used to calculate the following values: apparent volume of distribution (V_d), total steady-state volume of distribution (V_{ss}), central compartment volume (V_c), peripheral compartment volume (V_p), the apparent distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), area under the plasma concentration–time curve (AUC), and systemic total body clearance (CL). CL was determined using the following equation:

$$CL = F \times Dose/AUC \quad (3)$$

The bioavailability (*F*) of XN was determined for each oral dose by developing a systemic exposure profile obtained from measuring the concentration of free and conjugated XN over time in samples collected from the systemic circulation as defined by the Food and Drug Administration (FDA) (Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070124.pdf) [42] and using the following equation:

$$F = (AUC_{0-\infty}^{oraldose}/AUC_{0-\infty}^{i.v.}) \times (Dose^{i.v.}/Dose^{oraldose}) \quad (4)$$

Steady-state peak ($C_{ss,max}$) concentrations were estimated for the three oral doses (*D*) using the following equation with *D* = dose and τ = dosing interval = 24 h:

$$C_{ss,max} = (FD/V_d) \times (1/1 - e^{k\tau}) \times (e^{kT_{max}}) \quad (5)$$

.

3 Results and discussion

3.1 PK parameters of XN and its metabolites

The PK parameters for XN from IV (1.86 mg/kg) and three oral doses (1.86, 5.64, and 16.9 mg/kg) are listed in Table 1. Plasma concentration–time curves of XN are shown in Figs. 3 and 4. Following IV administration (Fig. 3), the plasma concentration–time profile of total XN demonstrated a biphasic decline. It started with a rapid distribution phase, where plasma concentration dropped to 50% in approximately 20 min, followed by a slow elimination phase up to 96 h. Extrapolation of the elimination phase yielded a $t_{1/2,\beta}$ of 33.78 ± 3.15 h. A low clearance rate (0.87 ± 0.14 L/h kg) and high steady-state volume of distribution (V_{ss}) (32.40 ± 6.16 L/kg) indicates high tissue distribution, which is also supported by observed large V_p values.

Following oral administration (Fig. 4), peak plasma XN levels were achieved at a similar T_{max} (~4 h post dosing) for low, medium, and high XN groups. High plasma concentrations of XN were observed between 0.5 and 2 h and 8 and 12 h, suggesting both small and large intestinal absorption as well as possible enterohepatic recirculation, as previously reported for other flavonoids [43–45]. The half-lives for the terminal phase also appear to be long for all oral groups (18–30 h).

Time courses for plasma concentrations of XN metabolites (IX and 8PN) after oral administration are shown in Figs. 5 and 6. There were barely detectable levels of 6PN in

Table 1. PK parameters for total free and conjugated XN for IV and oral treated rats ($n = 12$/group)

Parameter	Units	IV 1.86 mg/kg	Oral		
			1.86 mg/kg	5.64 mg/kg	16.9 mg/kg
A	mg/L	2.85±1.12	0.107±0.045	0.126±0.058	1.25±0.43
B	μg/L	21.2±3.3	4.46±1.28	10.5±6.6	15.1±12.9
k_a	h^{-1}	N/A	1.08±0.32	0.61±0.16	0.58±0.22
α	h^{-1}	2.09±0.20	0.40±0.26	0.10±0.01	0.15±0.04
β	h^{-1}	0.021±0.005	0.035±0.016	0.023±0.008	0.04±0.01
AUC	h* mg/L	2.54±0.34	0.839±0.168	1.03±0.12	2.49±0.10
Half-life $t_{1/2,\alpha}$ [a)]	h	0.33±0.03	1.75±0.38	7.18±1.16	4.55±0.97
Half-life $t_{1/2,\beta}$ [a)]	h	33.8±3.2	20.1±5.8	30.4±4.9	18.2±2.0
V_c/F	L/kg	1.16±0.19	72.8±12.1	88.2±5.7	96.0±51.2
V_p/F	L/kg	31.2±6.0	80.2±25.0	168±57	64.9±7.2
CL/F [a)]	$L\,h^{-1}\,kg^{-1}$	0.87±0.14	1.00±0.22	0.80±0.08	1.30±0.65
T_{max}	h	N/A	3.61±0.74	4.51±0.54	4.50±0.59
C_{max}	mg/L	2.87±0.11	0.019±0.002	0.043±0.002	0.15±0.01
V_{ss}	L/kg	32.4±6.2	50.6±8.6	34.2±7.4	17.4±5.4
F			0.33	0.13	0.11

All values are expressed as mean±SEM.
a) Values were calculated using harmonic means.

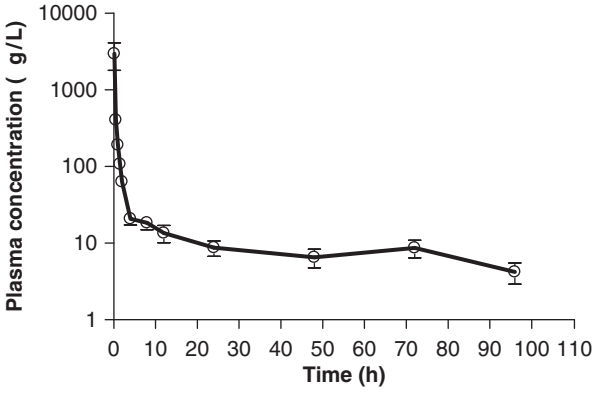

Figure 3. Semilogarithmic plot of mean plasma concentration–time data of xanthohumol obtained from Sprague–Dawley rats ($n = 12$) given a single intravenous injection dose of 1.86 mg/kg BW.

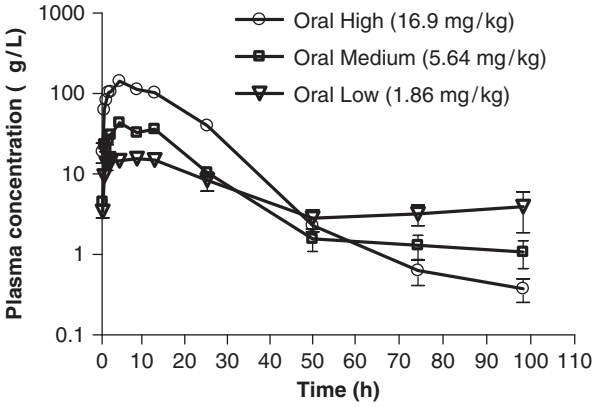

Figure 4. Semilogarithmic plot of mean plasma concentration–time data of xanthohumol obtained from Sprague–Dawley rats ($n = 12$/dose) given a single oral low, medium, or high dose (1.86, 5.64, or 16.9 mg/kg BW).

all treatment groups. Similar to XN, the elimination of IX also appears to be biphasic. For 8PN, however, the elimination process is adequately described with a one-compartment open model. The estimated PK parameters for IX and 8PN are detailed in Tables 2 and 3. There was also a greater amount of circulating XN metabolites compared with XN in oral medium- and high-dose animals at 24 and 48 h.

3.2 Hepatic demethylation of IX

The high amount of circulating IX in early time points (0.2–4 h) as well as 8PN at later time points (12–48 h) compared with barely detectable levels of 6PN suggests rapid isomerization of XN to IX followed by hepatic demethylation of IX to produce 8PN (Fig. 1). After an oral dose of XN, the peak plasma levels of IX reached a T_{max} around 7–8 h, whereas the T_{max} of 8PN is doubled (15–24 h). These findings are consistent with the current knowledge of the XN metabolic pathway (Fig. 1). Hepatic demethylation of IX to form 8PN may contribute to in vivo estrogenicity because 8PN has one of the most potent estrogenic activities among flavonoids [46–51]. It is generally believed that some health protective actions of flavonoids are due to their estrogenic activities. Previous studies have demonstrated that low micromolar concentrations of XN are associated with protective bone and heart health actions through stimulation of bone formation via osteoblast differentiation [52] and inhibition of LDL protein oxidation [53].

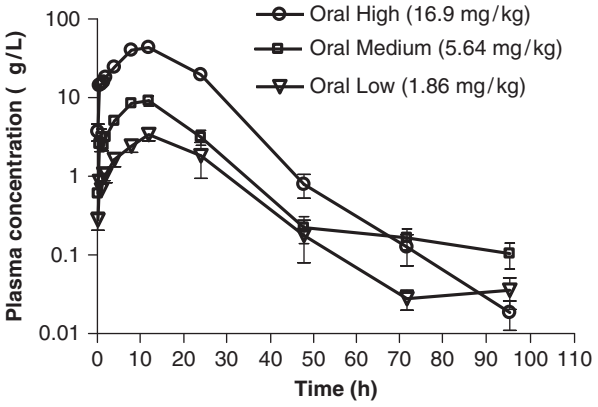

Figure 5. Semilogarithmic plot of mean plasma concentration–time data of isoxanthohumol obtained from Sprague–Dawley rats ($n = 12$/dose) given a single oral low, medium, or high dose (1.86, 5.64, or 16.9 mg/kg BW).

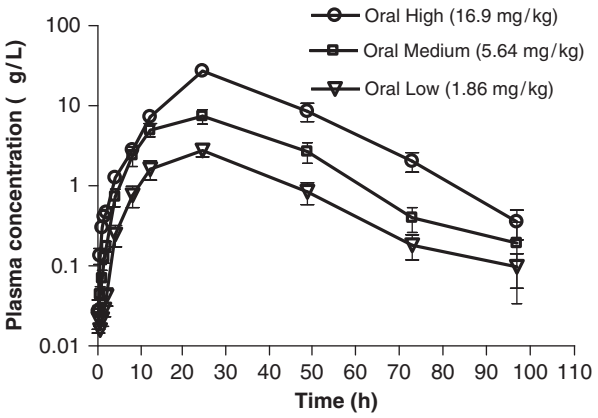

Figure 6. Semilogarithmic plot of mean plasma concentration–time data of 8-prenylnarigenin obtained from Sprague–Dawley rats ($n = 12$/dose) given a single oral low, medium, or high dose (1.86, 5.64, or 16.9 mg/kg BW).

3.3 Steady-state plasma concentrations

An oral low, medium, and high dose of XN resulted in projected steady-state peak concentrations ($C_{ss,max}$) of 0.094, 0.129, and 0.213 μM in rats. Preliminary data from our lab support this estimate. Daily feeding of the oral high XN dose (16.9 mg/kg BW per day), as a mixture of the oral gavage solution in 3 g of AIN 93G diet, to Sprague–Dawley rats ($n = 2$) resulted in plasma concentrations of 0.2–0.3 μM (data not shown).

3.4 Bioavailability

The bioavailability of total XN (free and conjugated) was calculated to be 33.1, 13.4, and 10.8% for low-, medium-, and high-dose groups, respectively. XN is similar, in terms of structure and biological activity, to soy isoflavones [54]; as expected, our findings on bioavailability are comparable to earlier works examining bioavailability of soy isoflavones. Qiu et al. determined the bioavailability of total daidzein (free and conjugated), a soy isoflavone, to be 47% for a low dose (20 mg/kg) in Wistar rats [55]. Both oral medium and high XN groups had similar XN bioavailability, which was lower than the oral low group suggesting that XN absorption in the gastrointestinal (GI) tract appears dependent on the dissolution of XN. Due to the low solubility of XN, bioavailability will decline as observed between oral low and medium doses. Similar dose–response effects on bioavailability have been observed with other flavonoids as well. Setchell et al. reported decreased bioavailability of soy isoflavones genistein and daidzein with increasing isoflavones intake in healthy women [56]. In addition to dose amount, dosage formulation of a flavonoid can also influence its bioavailability [55].

Table 2. PK parameters for total free and conjugated IX for IV and oral treated rats ($n = 12$/group)

Parameter	Units	IV 1.86 mg/kg	Oral		
			1.86 mg/kg	5.64 mg/kg	16.9 mg/kg
α	h^{-1}	1.45 ± 0.18	0.09 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
β	h^{-1}	0.11 ± 0.05	0.07 ± 0.03	0.08 ± 0.01	0.07 ± 0.01
AUC	h* μg/L	195 ± 41	79.3 ± 8.4	196 ± 26	820 ± 109
Half-life $t_{1/2,\alpha}$ [a)]	h	0.48 ± 0.06	8.1 ± 1.2	7.2 ± 2.4	8.4 ± 2.3
Half-life $t_{1/2,\beta}$ [a)]	h	6.4 ± 1.9	10.2 ± 3.9	8.6 ± 1.2	10.5 ± 3.7
CL/F [a)]	L h^{-1} kg^{-1}	13.8 ± 3.0	23.5 ± 2.9	28.7 ± 3.0	20.6 ± 1.9
T_{max}	h	N/A	7.81 ± 1.31	7.30 ± 1.00	8.14 ± 0.96
C_{max}	μg/L	81.1 ± 11.7	3.27 ± 0.29	8.15 ± 1.09	31.1 ± 4.4
V_{ss}	L/kg	312 ± 81	312 ± 84	393 ± 104	334 ± 86

All values are expressed as mean ± SEM.
a) Values were calculated using harmonic means.

Table 3. PK parameters for total free and conjugated 8-PN for IV and oral treated rats ($n = 12$/group)

Parameter	Units	IV 1.86 mg/kg	Oral		
			1.86 mg/kg	5.64 mg/kg	16.9 mg/kg
V/F	L/kg	499 ± 113	570 ± 124	654 ± 183	507 ± 67
k_a	h^{-1}	0.047 ± 0.003	0.077 ± 0.014	0.097 ± 0.033	0.042 ± 0.002
AUC	h* μg/L	121 ± 24	76.5 ± 12.4	284 ± 53	986 ± 169
Half-life, $t_{1/2}$	h	16.2 ± 1.7	9.8 ± 1.6	9.3 ± 2.9	16.6 ± 0.8
CL/F[a)]	$L\,h^{-1}\,kg^{-1}$	15.4 ± 3.2	24.3 ± 3.9	19.8 ± 5.8	17.1 ± 3.2
T_{max}	h	23.2 ± 1.4	17.2 ± 3.2	15.8 ± 1.9	24.3 ± 1.2
C_{max}	μg/L	2.2 ± 0.3	1.6 ± 0.2	5.1 ± 0.7	14.7 ± 2.2

All values are expressed as mean ± SEM.
a) Values were calculated using harmonic means.

4 Concluding remarks

Our findings on the fate of XN in rats and the dose-dependent bioavailability of XN provide pertinent information for subsequent studies. PK parameters obtained in this study can be utilized to predict steady-state plasma levels for XN and its metabolites at various dose levels and coupled with interspecies scaling could aid tremendously in determining the dosing regimen for clinical studies. Doses administered in this study (1.86, 5.64, 16.9 mg/kg BW) correspond to scaled values of 20, 60, 180 mg XN in humans with a BW of 66 kg. The most prominent flavonoid in beer is IX (due to isomerization of XN in brewery process) and the estimated daily intake of prenylflavonoids for the average American is 0.14 mg [16]. This suggests that dietary consumption alone will not be sufficient to attain health benefits of XN and highlights the need for future work to determine optimal therapeutic doses of XN. The dose-dependent bioavailability of XN observed in this study also emphasizes the importance of additional investigation of XN metabolism in order to elucidate and optimize the potential health benefits of XN and its metabolites.

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