Published in final edited form as:

Mol Nutr Food Res. 2012 March ; 56(3): 466–474. doi:10.1002/mnfr.201100554.

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

LeeCole Legette^{1,2}, Lian Ma², Ralph L. Reed^{1,2}, Cristobal L. Miranda^{1,2}, J. Mark Christensen², Rosita Rodriguez-Proteau², and Jan F. Stevens^{1,2}

¹Linus Pauling Institute, Oregon State University, Corvallis, Oregon, U.S.A

²College of Pharmacy, Oregon State University, Corvallis, Oregon, U.S.A.

Abstract

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 pharmacokinetic 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 ($AUC_{0-96\text{ h}}$) of total XN (free and conjugated) were 2.9 ± 0.1 mg/L and 2.5 ± 0.3 h*mg/L in the 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.

Keywords

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 antioxidant [3], an anti-inflammatory agent [4, 5], a cancer chemopreventive agent [6–13], a modulator of the immune system [14], an antimicrobial agent [15], and as an inhibitor of osteoporosis in post-menopausal women [16].

Correspondence: Dr. Jan Frederik Stevens, College of Pharmacy, 203 Pharmacy Building, 1601 SW Jefferson, Corvallis, OR 97331, U.S.A. **Phone:** +1-541-737-9534 **fred.stevens@oregonstate.edu** **Fax:** +1-541-737-3999.

Conflict of Interest The authors have declared no conflict of interest.

Recent evidence suggests that dietary bioactives could aid in 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 triglycerides 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, at concentrations in the range of 1–10 μ M. 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 triglyceride synthesis by 62% at 15 μ M and dose-dependently decreased apolipoprotein B secretion by 13 – 43 % at 5 – 15 μ M [26]. The decrease in triglyceride synthesis was attributed to 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 week 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 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) (Figure 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 μ mol/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 μ M (free XN), 0.11 μ M (conjugated XN), 1.04 μ M (free IX), and 4.87 μ M (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 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 IV dose level in order 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.

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 [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 Companies, Inc., St. Louis, MO, USA and checked for identity and purity (> 99%) by ¹H 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. After a 12 h fast, animals received a single oral gavage solution (1.86, 5.64, or 16.9 mg/kg BW). The animals in the IV group were given an intravenous injection (1.86 mg/kg BW) of XN dissolved in propylene glycol. 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 heparinized saline (20 U/mL). 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 Inc., Newton, NC, USA) and stored on ice immediately after collection. Samples were centrifuged for 10 min at 2000 × g (VWR Microcentrifuge 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 Company, Hillsborough, NJ) as internal standard, and treated with 600 U of *Helix pomatia* hydrolases dissolved in sodium acetate buffer (Sigma, St. Louis, MO) for 3 hours at 37 °C in a total volume of 600 µL to convert glucuronide and sulfate conjugates to their free aglycone forms. Incubation solutions were extracted three times with diethyl ether (1.0 mL) and centrifuged for 1 min at 8500 × g (VWR Microcentrifuge 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). 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, St. Louis, MO) 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.

2.5 Mass spectrometry

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), consisting of two LC-20AD pumps, a DQU-20A₅ 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) eluted with a gradient of 25 to 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. Parent → 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.

2.6 Pharmacokinetic model and parameters

Mean plasma concentration-time profiles for XN and its metabolites (IX, 6PN and 8PN) were generated using 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:

Oral administration:

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

Intravenous administration:

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

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:

$$CL = F \times \text{Dose} / \text{AUC} \quad (\text{Eq. 3})$$

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) [42] and using the following equation:

$$F = \left(\text{AUC}_{0-96}^{\text{oral dose}} / \text{AUC}_{0-96}^{\text{i.v.}} \right) \times \left(\text{Dose}^{\text{i.v.}} / \text{Dose}^{\text{oral dose}} \right) \quad (\text{Eq. 4})$$

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 = 24h:

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

3 Results and Discussion

3.1 Pharmacokinetic parameters of XN and its metabolites

The pharmacokinetic parameters for XN from IV (1.86 mg/kg) and three oral doses (1.86 mg/kg, 5.64 mg/kg, 16.9 mg/kg) are listed in Table 1. Plasma concentration-time curves of XN are shown in Figures 2 and 3. Following IV administration (Fig. 2), 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 minutes, 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 ($0.87 \pm 0.14 \text{ Lh}^{-1}\text{kg}^{-1}$) and high steady state volume of distribution (V_{ss}) ($32.40 \pm 6.16 \text{ L/kg}$) indicates high tissue distribution, which is also supported by observed large V_p values.

Following oral administration (Fig. 3), 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 – 2 h and 8 – 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 Figures 4 – 5. There were barely detectable levels of 6PN in 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 pharmacokinetic parameters for IX and 8PN are detailed in Tables 2 – 3. There was also a greater amount of circulating XN metabolites compared to 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 to 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 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].

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 supports 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 %, 13 % and 11 % 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 a saturation effect occurs 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].

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. Pharmacokinetic 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.

Acknowledgments

Support from the National Institutes of Health (R21AT005294, S10 RR022589 and P30 ES000210), USANA Health Sciences, Inc., Salt Lake City, UT, Anheuser-Busch Companies, Inc., St. Louis, MO, and Hopsteiner Inc., New York, is gratefully acknowledged. We thank Mr. Jeffrey Morré for technical assistance.

Abbreviations

6PN	6-prenylnaringenin
8PN	8-prenylnaringenin
AIN93G	1993 American Institute of Nutrition dietary guidelines for growing rodents
AUC	area under the plasma concentration time curve
BW	body weight
C_{max}	maximum plasma concentration
C_{ss,max}	steady state peak concentrations
C_{ss,min}	steady state trough concentrations
CL	systematic total body clearance
DHC	4,2'-dihydroxychalcone
FDA	Food and Drug Administration
IV	intravenous
IX	isoxanthohumol
k_a	absorption rate constant
PK	pharmacokinetics
SRM	selective reaction monitoring
t_{1/2, α}	half-life of distribution phase
t_{1/2, β}	half-life of elimination phase
T_{max}	time required to reach maximum plasma concentration
V_c	central compartment volume
V_d	apparent volume of distribution
V_p	peripheral compartment volume
V_{ss}	total steady state volume of distribution
XN	xanthohumol

5 References

- [1]. Stevens JF, Ivancic M, Hsu V, Deinzer ML. Prenylflavonoids from *Humulus lupulus*. *Phytochemistry*. 1997; 44:1575–1585.
- [2]. Stevens JF, Taylor AW, Deinzer ML. Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. *J Chromatogr A*. 1999; 832:97–107. [PubMed: 10070768]
- [3]. Miranda CL, Stevens JF, Ivanov V, McCall M, et al. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. *J Agric Food Chem*. 2000; 48:3876–3884. [PubMed: 10995285]
- [4]. Lupinacci E, Meijerink J, Vincken JP, Gabriele B, et al. Xanthohumol from hop (*Humulus lupulus* L.) is an efficient inhibitor of monocyte chemoattractant protein-1 and tumor necrosis factor- α release in LPS-stimulated RAW 264.7 mouse macrophages and U937 human monocytes. *J Agric Food Chem*. 2009; 57:7274–7281. [PubMed: 19634869]
- [5]. Peluso MR, Miranda CL, Hobbs DJ, Proteau RR, Stevens JF. Xanthohumol and related prenylated flavonoids inhibit inflammatory cytokine production in LPS-activated THP-1 monocytes:

structure-activity relationships and in silico binding to myeloid differentiation protein-2 (MD-2). *Planta Med.* 2010; 76:1536–1543. [PubMed: 20309792]

- [6]. Colgate EC, Miranda CL, Stevens JF, Bray TM, Ho E. Xanthohumol, a prenylflavonoid derived from hops induces apoptosis and inhibits NF-kappaB activation in prostate epithelial cells. *Cancer Lett.* 2007; 246:201–209. [PubMed: 16563612]
- [7]. Miranda CL, Stevens JF, Helmrich A, Henderson MC, et al. Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupulus*) in human cancer cell lines. *Food Chem Toxicol.* 1999; 37:271–285. [PubMed: 10418944]
- [8]. Miranda CL, Aponso GL, Stevens JF, Deinzer ML, Buhler DR. Prenylated chalcones and flavanones as inducers of quinone reductase in mouse Hepa 1c1c7 cells. *Cancer Lett.* 2000; 149:21–29. [PubMed: 10737704]
- [9]. Miranda CL, Yang YH, Henderson MC, Stevens JF, et al. Prenylflavonoids from hops inhibit the metabolic activation of the carcinogenic heterocyclic amine 2-amino-3-methylimidazo[4, 5-f]quinoline, mediated by cDNA-expressed human CYP1A2. *Drug Metab Dispos.* 2000; 28:1297–1302. [PubMed: 11038156]
- [10]. Plazar J, Filipic M, Groothuis GM. Antigenotoxic effect of Xanthohumol in rat liver slices. *Toxicol In Vitro.* 2008; 22:318–327. [PubMed: 17981005]
- [11]. Gerhauser C. Beer constituents as potential cancer chemopreventive agents. *Eur J Cancer.* 2005; 41:1941–1954. [PubMed: 15953717]
- [12]. Gerhauser C, Alt A, Heiss E, Gamal-Eldeen A, et al. Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. *Mol Cancer Ther.* 2002; 1:959–969. [PubMed: 12481418]
- [13]. Dietz BM, Kang YH, Liu G, Egger AL, et al. Xanthohumol isolated from *Humulus lupulus* inhibits menadione-induced DNA damage through induction of quinone reductase. *Chem Res Toxicol.* 2005; 18:1296–1305. [PubMed: 16097803]
- [14]. Xuan NT, Shumilina E, Gulbins E, Gu S, et al. Triggering of dendritic cell apoptosis by xanthohumol. *Mol Nutr Food Res.* 2010; 54(Suppl 2):S214–224. [PubMed: 20333722]
- [15]. Gerhauser C. Broad spectrum anti-infective potential of xanthohumol from hop (*Humulus lupulus* L.) in comparison with activities of other hop constituents and xanthohumol metabolites. *Mol Nutr Food Res.* 2005; 49:827–831. [PubMed: 16092071]
- [16]. Stevens JF, Page JE. Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry.* 2004; 65:1317–1330. [PubMed: 15231405]
- [17]. Ferrari CK. Functional foods, herbs and nutraceuticals: towards biochemical mechanisms of healthy aging. *Biogerontology.* 2004; 5:275–289. [PubMed: 15547316]
- [18]. Heber D. Vegetables, fruits and phytoestrogens in the prevention of diseases. *J Postgrad Med.* 2004; 50:145–149. [PubMed: 15235216]
- [19]. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med.* 2002; 113(Suppl 9B):71S–88S. [PubMed: 12566142]
- [20]. Milner JA. Molecular targets for bioactive food components. *J Nutr.* 2004; 134:2492S–2498S. [PubMed: 15333748]
- [21]. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA.* 2002; 287:356–359. [PubMed: 11790215]
- [22]. Nozawa H. Xanthohumol, the chalcone from beer hops (*Humulus lupulus* L.), is the ligand for farnesoid X receptor and ameliorates lipid and glucose metabolism in KK-A(y) mice. *Biochem Biophys Res Commun.* 2005; 336:754–761. [PubMed: 16140264]
- [23]. Mendes V, Monteiro R, Pestana D, Teixeira D, et al. Xanthohumol influences preadipocyte differentiation: implication of antiproliferative and apoptotic effects. *J Agric Food Chem.* 2008; 56:11631–11637. [PubMed: 19035642]
- [24]. Rayalam S, Yang JY, Della-Fera MA, Park HJ, et al. Anti-obesity effects of xanthohumol plus guggulsterone in 3T3-L1 adipocytes. *J Med Food.* 2009; 12:846–853. [PubMed: 19735186]
- [25]. Yang JY, Della-Fera MA, Rayalam S, Baile CA. Effect of xanthohumol and isoxanthohumol on 3T3-L1 cell apoptosis and adipogenesis. *Apoptosis.* 2007; 12:1953–1963. [PubMed: 17874298]

- [26]. Casaschi A, Maiyoh GK, Rubio BK, Li RW, et al. The chalcone xanthohumol inhibits triglyceride and apolipoprotein B secretion in HepG2 cells. *J Nutr.* 2004; 134:1340–1346. [PubMed: 15173394]
- [27]. Dorn C, Kraus B, Motyl M, Weiss TS, et al. Xanthohumol, a chalcone derived from hops, inhibits hepatic inflammation and fibrosis. *Mol Nutr Food Res.* 54(Suppl 2):S205–213. [PubMed: 20087858]
- [28]. Guo J, Nikolic D, Chadwick LR, Pauli GF, van Breemen RB. Identification of human hepatic cytochrome P450 enzymes involved in the metabolism of 8-prenylnaringenin and isoxanthohumol from hops (*Humulus lupulus* L.). *Drug Metab Dispos.* 2006; 34:1152–1159. [PubMed: 16611861]
- [29]. Possemiers S, Bolca S, Grootaert C, Heyerick A, et al. The prenylflavonoid isoxanthohumol from hops (*Humulus lupulus* L.) is activated into the potent phytoestrogen 8-prenylnaringenin in vitro and in the human intestine. *J Nutr.* 2006; 136:1862–1867. [PubMed: 16772450]
- [30]. Possemiers S, Heyerick A, Robbens V, De Keukeleire D, Verstraete W. Activation of proestrogens from hops (*Humulus lupulus* L.) by intestinal microbiota; conversion of isoxanthohumol into 8-prenylnaringenin. *J Agric Food Chem.* 2005; 53:6281–6288. [PubMed: 16076107]
- [31]. Bolca S, Possemiers S, Maervoet V, Huybrechts I, et al. Microbial and dietary factors associated with the 8-prenylnaringenin producer phenotype: a dietary intervention trial with fifty healthy post-menopausal Caucasian women. *Br J Nutr.* 2007; 98:950–959. [PubMed: 17521469]
- [32]. Yilmazer M, Stevens JF, Buhler DR. In vitro glucuronidation of xanthohumol, a flavonoid in hop and beer, by rat and human liver microsomes. *FEBS Lett.* 2001; 491:252–256. [PubMed: 11240137]
- [33]. Yilmazer M, Stevens JF, Deinzer ML, Buhler DR. In vitro biotransformation of xanthohumol, a flavonoid from hops (*Humulus lupulus*), by rat liver microsomes. *Drug Metab Dispos.* 2001; 29:223–231. [PubMed: 11181488]
- [34]. Jirasko R, Holcapek M, Vrublova E, Ulrichova J, Simanek V. Identification of new phase II metabolites of xanthohumol in rat in vivo biotransformation of hop extracts using high-performance liquid chromatography electrospray ionization tandem mass spectrometry. *J Chromatogr A.* 2010
- [35]. Nikolic D, Li Y, Chadwick LR, Grubjesic S, et al. Metabolism of 8-prenylnaringenin, a potent phytoestrogen from hops (*Humulus lupulus*), by human liver microsomes. *Drug Metab Dispos.* 2004; 32:272–279. [PubMed: 14744951]
- [36]. Nookandeh A, Frank N, Steiner F, Ellinger R, et al. Xanthohumol metabolites in faeces of rats. *Phytochemistry.* 2004; 65:561–570. [PubMed: 15003419]
- [37]. Hanske L, Loh G, Sczesny S, Blaut M, Braune A. Recovery and metabolism of xanthohumol in germ-free and human microbiota-associated rats. *Mol Nutr Food Res.* 2010; 54:1405–1413. [PubMed: 20397197]
- [38]. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr.* 1993; 123:1939–1951. [PubMed: 8229312]
- [39]. [Accessed on August 2, 2011] Food and Drug Administration, Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. 2005. Available at: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078932.pdf>.
- [40]. Stevens JF, Taylor AW, Clawson JE, Deinzer ML. Fate of xanthohumol and related prenylflavonoids from hops to beer. *J Agric Food Chem.* 1999; 47:2421–2428. [PubMed: 10794646]
- [41]. Ludden TM, Beal SL, Sheiner LB. Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection. *J Pharmacokinet Biopharm.* 1994; 22:431–445. [PubMed: 7791040]
- [42]. [Accessed on August 2, 2011] Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations. 2003. Available at: <http://www.fda.gov/cder/rdmt/oc/guidance.htm>

www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070124.pdf.

- [43]. Chen J, Lin H, Hu M. Metabolism of flavonoids via enteric recycling: role of intestinal disposition. *J Pharmacol Exp Ther*. 2003; 304:1228–1235. [PubMed: 12604700]
- [44]. Jia X, Chen J, Lin H, Hu M. Disposition of flavonoids via enteric recycling: enzyme-transporter coupling affects metabolism of biochanin A and formononetin and excretion of their phase II conjugates. *J Pharmacol Exp Ther*. 2004; 310:1103–1113. [PubMed: 15128864]
- [45]. Sfakianos J, Coward L, Kirk M, Barnes S. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *J Nutr*. 1997; 127:1260–1268. [PubMed: 9202077]
- [46]. Bovee TF, Helsdingen RJ, Rietjens IM, Keijer J, Hoogenboom RL. Rapid yeast estrogen bioassays stably expressing human estrogen receptors alpha and beta, and green fluorescent protein: a comparison of different compounds with both receptor types. *J Steroid Biochem Mol Biol*. 2004; 91:99–109. [PubMed: 15276617]
- [47]. Coldham NG, Sauer MJ. Identification, quantitation and biological activity of phytoestrogens in a dietary supplement for breast enhancement. *Food Chem Toxicol*. 2001; 39:1211–1224. [PubMed: 11696395]
- [48]. Kitaoka M, Kadokawa H, Sugano M, Ichikawa K, et al. Prenylflavonoids: a new class of non-steroidal phytoestrogen (Part 1). Isolation of 8-isopentenylnaringenin and an initial study on its structure-activity relationship. *Planta Med*. 1998; 64:511–515. [PubMed: 9741295]
- [49]. Milligan S, Kalita J, Pocock V, Heyerick A, et al. Oestrogenic activity of the hop phyto-oestrogen, 8-prenylnaringenin. *Reproduction*. 2002; 123:235–242. [PubMed: 11866690]
- [50]. Milligan SR, Kalita JC, Heyerick A, Rong H, et al. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *J Clin Endocrinol Metab*. 1999; 84:2249–2252. [PubMed: 10372741]
- [51]. Milligan SR, Kalita JC, Pocock V, Van De Kauter V, et al. The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids. *J Clin Endocrinol Metab*. 2000; 85:4912–4915. [PubMed: 11134162]
- [52]. Jeong HM, Han EH, Jin YH, Choi YH, et al. Xanthohumol from the hop plant stimulates osteoblast differentiation by RUNX2 activation. *Biochem Biophys Res Commun*. 409:82–89. [PubMed: 21565172]
- [53]. Stevens JF, Miranda CL, Frei B, Buhler DR. Inhibition of peroxynitrite-mediated LDL oxidation by prenylated flavonoids: the alpha,beta-unsaturated keto functionality of 2'-hydroxychalcones as a novel antioxidant pharmacophore. *Chem Res Toxicol*. 2003; 16:1277–286. [PubMed: 14565769]
- [54]. Overk CR, Yao P, Chadwick LR, Nikolic D, et al. Comparison of the in vitro estrogenic activities of compounds from hops (*Humulus lupulus*) and red clover (*Trifolium pratense*). *J Agric Food Chem*. 2005; 53:6246–6253. [PubMed: 16076101]
- [55]. Qiu F, Chen XY, Song B, Zhong DF, Liu CX. Influence of dosage forms on pharmacokinetics of daidzein and its main metabolite daidzein-7-O-glucuronide in rats. *Acta Pharmacol Sin*. 2005; 26:1145–1152. [PubMed: 16115384]
- [56]. Setchell KD, Brown NM, Desai PB, Zimmer-Nechimias L, et al. Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *J Nutr*. 2003; 133:1027–1035. [PubMed: 12672914]

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

Figure 2. Semilogarithmic plot of mean plasma concentration–time data of XN obtained from Sprague Dawley rats ($n = 12$) given a single intravenous dose of 1.86 mg / kg BW.

Figure 3. Semilogarithmic plot of mean plasma concentration–time data of XN obtained from Sprague Dawley rats ($n = 12/\text{dose}$) given a single oral dose of XN (1.86, 5.64 or 16.9 mg / kg BW).

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

Figure 5. Semilogarithmic plot of mean plasma concentration–time data of 8PN obtained from Sprague Dawley rats ($n = 12/\text{dose}$) given a single oral dose of XN (1.86, 5.64 or 16.9 mg / kg BW).

Pharmacokinetic (PK) parameters for total free and conjugated XN after treating rats with a single IV or oral dose of XN (n = 12/group)

Table 1

Parameter	Units	IV		Oral		
		1.86 mg/kg	1.86 mg/kg	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 ^{a)} t _{1/2,α}	h	0.33 ± 0.03	1.75 ± 0.38	7.18 ± 1.16	4.55 ± 0.97	
half-life ^{a)} t _{1/2,β}	h	33.8 ± 3.2	20.1 ± 5.8	30.4 ± 4.9	18.2 ± 2.0	
V _d /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.

Table 2

Pharmacokinetic parameters for total free and conjugated IX after treating rats with a single IV or oral dose of XN (n = 12/group)

Parameter	Units	IV		Oral		
		1.86 mg/kg	1.86 mg/kg	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	0.08 ± 0.01
β	h^{-1}	0.11 ± 0.05	0.07 ± 0.03	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
AUC	$\text{h}^* \mu\text{g/L}$	195 ± 41	79.3 ± 8.4	196 ± 26	820 ± 109	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	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	10.5 ± 3.7
CL/F ^{a)}	$\text{L h}^{-1} \text{kg}^{-1}$	13.8 ± 3.0	23.5 ± 2.9	28.7 ± 3.0	20.6 ± 1.9	20.6 ± 1.9
$T_{\max}$	h	N/A	7.81 ± 1.31	7.30 ± 1.00	8.14 ± 0.96	8.14 ± 0.96
$C_{\max}$	$\mu\text{g/L}$	81.1 ± 11.7	3.27 ± 0.29	8.15 ± 1.09	31.1 ± 4.4	31.1 ± 4.4
V_{ss}	L/kg	312 ± 81	312 ± 84	393 ± 104	334 ± 86	334 ± 86

All values are expressed as mean $\pm$ SEM.

^{a)}Values were calculated using harmonic means.

Table 3

Pharmacokinetic parameters for total free and conjugated 8PN after treating rats with a single IV or oral dose of XN (n = 12/group)

Parameter	Units	IV		Oral	
		1.86 mg/kg	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.